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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT) (51) International Patent Classification 6 : (11) International Publication Number: WO 97J32885	C07D 487/22 A1 (43) International Publication Date: 12 September 1997 (12.09.97) (21) International Application Number: PCT/US97/03891 (81) Designated States: CA, JP, MX, European patent (AT, BE, C3) International Filing Date: 7 March 1997 (07.03.97) PT, SE).	 (30) Priority Data: (30) Priority Data: (31) Applicant: HEALTH RESEARCH, 197 (06,03.97) (71) Applicant: HEALTH RESEARCH, INC. [US/US]; Roswell (71) Inventors: PANDEY, Ravindra, K.: 75 Lemay Court, Williamsville. NY 14250 (US). (72) Inventors: PANDEY, Ravindra, K.: 75 Lemay Court, Williamsville. NY 14221 (US). (73) Agent. DVN, Michael L.: Dunn & Associates, P.O. Box 96. 	T T T T T T T T T T T T T T T T T T T		e مرجور) برا محمد مرد مرد مرد مرد مرد مرد مرد مرد مرد م

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SYNTHESIS OF ISOIMIDE OF CHLORINS AND BACTERIOCHLORINS AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF CANCER

Background of the Invention

This invention relates to treatment and diagnosis of cancer through the use of IR imaging and photodynamic therapy employing porphyrin related compounds and more particularly certain analogs of chlorins and bacteriochlorins.

Most. The these active substances in higher concentrations and and/or retention time is not dependent on whether or not the cells are synthesizing DNA or undergoing cell growth or nutrient uptake. for longer durations than surrounding normal tissues. fluoresce when activated by light of a specific wavelength. Photosensitizers are chemicals which kill cells retain tissues premalignant some and photochemically malignant

Light sensitive drugs, lasers and fiber optic probes have been combined in a procedure known as photodynamic therapy (PDT). PDT has emerged as one of the most promising strategies in cancer treatment (including cancer detection). PDT is being increasingly used where chemotherapy, surgery and high energy irradiation have failed. In this new therapy, patients are given intravenous injections of a photodynamic drug that accumulates in cancer cells in much higher concentrations than in normal cells. The photodynamic (photosensitizing) drug is then activated to

too frail to therapy, which often requires just local anesthesia and does not necessitate hospital cancer cells tolerate the stress of major surgery, chemotherapy or high energy admission. PDT is an important form of cancer therapy that has many additional advantages, e.g. it can be performed any number of times on a single patient, it is not contraindicated with other cancer therapies and it allows selective treatment of malignant tissues due to preferential retention of dye in cancer <u>Р</u> early cells and it has already been established that for kill the cancer cells by a laser beam directed to <u>ч</u> too old superficial malignancy, PDT may be curative. photodynamic People who are be helped by through fiber optics. radiation may

therapeutic use of photosensitizers when he used eosin and white The C., Z. Biol., 1900, 39, 1423) reported the lethal effects of a combination of acridin orange dye and ordinary light localization of administered porphyrins in tumor tissue was that these two ideas (photodegradation of tissue and localization in came together successfully, when Diamond demonstrated that a porphyrin could preferentially degrade tumor implants in a Photosensitizers have been recognized for almost a century. of an rat (Diamond, I.; McDonagh, A.F.; Wilson, C.B.; Granelli, S.G.; Nielsen, S.; Jaenicke, R., Lancet, 1972, 1175). This result was first Dougherty, T.J.; Grindey, G.B.; Fiel, was not until 1972, however, In 1903, von Tappeneir reported the 1913. The phototoxic effect administered porphyrin in man was observed in to treat skin tumors. цt confirmed and extended by recognized in the 1940's. on Paramecium. In 1900, (Rabb, tumors) light

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R.; Weishaupt, K.R.; Boyle, D.G.; J. Natl. Cancer Inst., 1975, 55, 115. The higher concentration of porphyrins in malignant tumors is used for the treatment and detection of cancer. For detection of early stage small tumors, the porphyrin-containing tumor cells The porphyrins then emit a strong fluorescence, which contrasts with the much tissue, allowing for of cancer, photodynamic therapy consists of injecting the patient with a photoactive dye The and irradiating the tumor area with a wavelength of light which producing single oxygen and oxygen radicals (Dougherty, T.J.; to the surrounding environment by PDT techniques depend strongly on how well the compound used preferentially is the of PDT with certain porphyrin type avoid photosensitizers. Because skin retains these chemicals in enough Kaufman, J.H.; Goldfarb, A.; Weishaupt, K.R.; Boyle, activates the dye to produce toxins which kill the tumor. produce surface reactions, patients must Skin photosensitivity Mittleman, A.; *Cancer Res.*, 1976, 38, 3628). and surrounding tissues are exposed to light. weaker fluorescence from the normal concentrates within the tumor cell. For the treatment porphyrin dyes become toxic only known side effect exposure to sunlight. quantities to detection. (PDT)

The distribution of porphyrin drugs in the body compared with tumor cells is still under investigation. The distribution varies with cell type and porphyrin derivative. It is thought that once the photosensitizer is injected intravenously, some of

porphyrin binds to the cellular membrane and slowly the drug escapes the blood stream and moves into the interstitial Each porphyrin, then, rapidly Fluorescence cells shows a and within the to hydrophobic regions inside the cell. porphyrin-treated leukemia L1210 membrane plasma cytoplasm. the diffuses into the cell localization around intracellular vesicles. microscopy of The fluid. binds

Photofrin $(oldsymbol{\$})$ a hematoporphyrin derivative (Dougherty, T.J.; Boyle, D.G.; Weishaupt, K.R., "Photodynamic Therapy - Clinical is the only photosensitizer currently being used all over the world for the treatment of a variety of solid Hpd thus produced consists of a variety of porphyrins. When gel filtration Т.Ј.; The main components of Photofrin() are dimers and higher oligomers linked with ether, and possibly carbon-carbon linkages J.M.; Press, mixing hematoporphyrin with glacial acetic acid and sulfuric acid, called The recommended human dosage of Photofrin® is 1-2 mg/kg of body followed by hydrolysis and precipitation under acidic conditions. This method was partially described by Lipson et al (Lipson, R.L.; Baldes, E.J.; Olsen, A.M., J. Natl. Cancer Inst., 1961, 26, Bellnier, D.A.; Wityk, K.E., Adv. Exp. Biol. Med., 1983, 160, 3). B.; Potter, and Drug Advances, Porphyrin Photosensitization," Plenum Hematoporphyrin derivative (Hpd) is prepared by agent (Dougherty, with Sephadex LH-20, the higher molecular weight portion, R.; McReynolds, is separated into its two main fractions by Boyle, D.G.; Weishaupt, K.R.; Henderson, M.M.; Tsao, PDT Photofrin®, is a more efficient (Pandey, R.K.; Siegel, New York, 1983, p. 3) weight. tumors. . ה рdн

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Dougherty, T.J., Biomed. and Environ. Mass Spectrometry, 1990, 19, 405).

a photosensitizer to be clinically useful, it must be non-toxic, selectively taken up and/or retained in malignant nm), and photochemically efficient. Although Photofrin® has been approved for commercialization in Canada, Europe and the United States, it H Ē of porphyrin oligomers, and has the disadvantage that its absorbance at 630 complex mixture improvement New tissues, activated by penetrating light (>600 the penetration. lacks rapid clearance from tissues, is a photosensitizers are thus needed for photodynamic therapy for cancer treatment. tissue for optimized For is not

There is a need for more efficient, chemically pure, less Inst., 1988, 80, 330). With these compounds, the aspartyl group e_6 , monoaspartyl chlorin e_6 and diaspartyl chlorin e_6 , were found noted to be responsible for the efficiency of tissue hexyloxyethyl)2-des vinyl derivatives were found to be excellent phototoxic, and better localizing porphyrins. Important prior chlorin F.Y.; Nelson, J.S.; Smith, K.M., Roberts, M.W., J. Natl. Cancer clearance. In pheophorbide, pyropheophorbide and chlorin e₆ art porphyrin and chlorin derivatives have been reviewed by methyl Pandey, R.K.; Majchrzycki, D.F.; Smith, K.M.; Dougherty, T.J., be effective photosensitizers in vitro (Roberts, W.G.; Shaiu, series, certain alkyl ether derivatives including 2-(1-The aspartyl derivatives of compounds, parent compared with *Proc. SPIE*, 1989, 1065, 104. photosensitizers was ß

pheophorbide-a, pyropheophorbide- and chlorin₆. (Pandey, R.K.; Bellnier, D.A.; Smith, K.M.; Dougherty, T.J., Photochem. Photobiol., 1991, 53, 65). This was attributed to the increased hydrophobicity of the hexyl group and is consistent with studies done by Evenson on porphyrins with varying polarities (Evenson, J.F.; Sommer, S.; Riminfton, C.; Moan, J., Br. J. Cancer, 1987, 55, 483).

chlorins, on This methodology has been extended in the pheophorbide-a and chlorin e₆ series, by preparation at a reacting with osmium tetroxide can be converted to vic dihydroxy Chem. series of vic -dihydroxy and keto-bacteriochlorins (Pandey, R.K.; & Med. Chem. Lett., 1992, 2, 491). It has also been reported that the regiospecificity of pyrrole subunits in osmium tetroxide electron bacteriochlorins, prepared from mesochlorin e₆ trimethylester and pyropheophorbide-a methylester, have strong absorption in the red region (730 to 750 nm), but, did not show any significant in vivo stable photosensitizing activity (Kessel, D.; Smith, K.M.; Pandey, R.K.; Shiau, F.Y.; Sumlin, A.B.; Dougherty, T.J.; Smith, K.M., Bioorg. 58, Soc., F.Y.; Henderson, B., Photochem. Photobiol., 1993, These oxidation is affected significantly by the presence of Chang, C.K., Sotiroiu, C.; Wu, W., J. Chem. that withdrawing substituents in the macrocycle(5a). Commun., 1986, 1213, have previously shown bacteriochlorin system. Shaiu, 200).

Hoober, J.K.; Sery, T.W.; Yamamoto, Y., Photochem. Photobiol., 1988, 48, 579 showed that purpurin-18 2, which has

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strong absorption at 700 nm might be a useful photosensitizer for	
photodynamic therapy (PDT).	compound 8) have in the past been prepared by cyclizing chlorin-
Among long wavelength absorbing photosensitizers,	P ₆ 6-N-hexylamide-7-methyl ester 3A (compound 3 reacted with
bacteriochlorins have been proposed as potential useful	diazomethane). The resulting cyclized reaction mixture contains
candidates for use in photodynamic therapy (PDT) where strong	a number of products in addition to the cyclic amine, especially
absorptions in the visible spectrum can be used to photoactivate	unreacted starting material 3A. The synthesis route therefore is
dyes previously located in targeted (neoplastic) tissues (Pandey,	not only inefficient because of low yield, but requires
R.K.; Shiau, F.Y.; Isaac, M.; Ramaprasad, S.; Dougherty, T.J.;	significant subsequent purification. Further such compounds have
Smith, K.M., Tetrahedron Lett., 1992, 33, 7815). Some naturally	not had optimal hydrophylic-lipophylic balance.
occurring bacteriochlorins, have previously been reported as	<u>Brief Description of the Drawings</u>
effective photosensitizers both in vitro and as in vivo (Beems,	Figure 1 is a schematic equation showing the degeneration of
E.M.; Dubbelman, T.M.A.R.; Lugtenburg, J.; Best, J.A.B.; Smeets,	
M.F.M.A.; Boehgeim, J.P.J., Photochem. Photobiol., 1987, 46,	Figure 2 is a schematic equation showing the synthetic route
639). However, most of the naturally occurring bacteriochlorins	
(760-780 nm) are extremely sensitive to oxygen, which results in	
rapid oxidation to the chlorin state (640 nm); thus the	is a schemat
spectroscopic properties of the bacteriochlorins are lost.	
Further, if a laser is used to excite the bacteriochlorin in	
vivo, oxidation may result in the formation of a new chromophore	9
absorbing outside the laser window, thus reducing the	Figure 5 is a schematic equation showing the synthetic route
photodynamic efficiency.	ntion.
It has been found that certain cyclic amide derivatives of	Figure 6A is a reflection spectroscopy curve showing
porphyrins, including bacteriochlorins and chlorins, have both an	im revo
increased wavelength and the requisite stability to meet the	owing the ratio of timer over mid
reguirements of an improved photodynamic therapeutic agent.	as renr
Unfortunately, the preparation of such compounds is difficult and	
yields are very low, e.g. 10 to 30%. Such compounds (e.g.	
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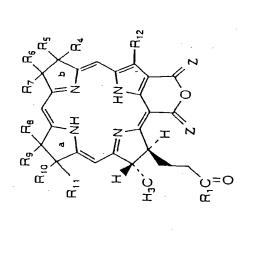
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Brief Description of the Invention

In accordance with the present invention, there are provided new chlorin and bacteriochlorin derivatives having utility as fluorescent and photosensitizing compounds. Such compounds may be excited by microwaves, ultrasound, and visible or infrared radiation.

All of such novel compounds described herein may be used in traditional areas where compounds having such properties have utility. The compounds, may, for example, be incorporated into a substance such as a plastic product, excited with ultrasound, microwaves or visible light followed by using known methods for detecting emitted radiation to image the product for the purpose of detecting voids or other flaws in the product. Certain of such compounds have special utility as photosensitizers in the area of photodynamic therapy for the detection and treatment of tumors. In accordance with the invention, to make PDT more applicable, there is a need of long wavelength absorbing photosensitizers such as stable bacteriochlorins which have the ability to localize in high concentration at the tumor site. Furthermore there is a need for an efficient and cost effective method for preparing such photosensitizers.

In accordance with the invention, a compound is therefore provided which comprises a chemical of the formula:



ģ polyamine group, a polyether group or OR₁₃ where R₁₃ is alkyl; $^{-OR_{16}}$, where R_{16} is H, alkyl or aryl, or a carbonyl containing or an group, provided that; R_4 may be taken together with R_5 to form amino acid group; ${f R}_4$ through ${f R}_{11}$ are -H, -OH, alkyl, alkylene, =0; R_6 may be taken together with R_7 to form =0; R_8 may be taken together with $extsf{R}_9$ to form =0; $extsf{R}_{10}$ may be taken together with $extsf{R}_{11}$ to form =0; and R_4 and R_7 may together form a chemical bond and ${
m R}_{
m g}$ and ${
m R}_{
m l}$ may together form a chemical bond; and ${
m R}_{
m l2}$ is hydrogen is. ■ NR₁₄; R₁ is an amino acid group, ы or lower alkyl; provided that if one z is =0, the other ^R14 is alkyl, or substituted alkyl, a polyamine group, **ы** 0 N wherein z is =NR₁₄.

The invention further includes a method for using the above compound as an intermediate for the preparation of additional

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long wave length stable photosensitizers and a method for its	it to the subsequent reactions described herein. The anhydride
preparation.	is then reacted with 1, hexyl amine to open the anhydride ring,
<u>Detailed Description of the Invention</u>	e.g., as shown in Figure 2 to obtain 6 carboxylhexyl amide 2 and
The invention permits more flexibility in the preparation of	γ -carboxy-hexylamide J.
porphyrin-type compounds than was previously possible.	The 6 carboxyhexylamide and the $\gamma ext{-carboxyhexylamide}$ are then
Intermediate compounds may be provided with a number of	reacted separately or together with a carbodiimide to form a
substituents on the a and b rings and variable substituents at	porphyrin diimide which is unstable and immediately converts to
R1.	an isoimid, the compound of the invention. A preferred
The a and b rings may be saturated or unsaturated at the ${ t R}_a-$	carbodiimide is dicyloberylcarbodiimide (DCC), which results in
R ₁₁ positions or may contain hydrogen, hydroxy, formal or	compounds similar to 6 and 7 in Figure 2 where the ${ m R_1-R_{12}}$
substituted and unsubstituted alkyl, alkoxy, alkenyl, aryl, and	substituents may vary as described herein.
aryloxy groups. The alkyl, alkoxy, alkenyl, aryl and aryloxy	The invention may be described in more detail by reference
groups usually contain 1 through 8 carbon atoms and more commonly	to the following specific embodiment.
contain 1 through 3 carbon atoms. A limited number, i.e., as	Initially, in order to establish the reaction conditions,
many as 2, of such carbon containing groups may be long chain	purpurin-18 methyl ester 1 was used as the starting material. As
carbon containing groups, e.g., up to 22 carbon atoms.	expected, reaction of 1 (Amax 700 nm) with 1-hexylamine gave the
The carbon containing groups may be substituted with	corresponding amides in 95% yield as a mixture of 2 and 3 in the
carbonyl, hydroxy, phosphoro, carboxy, halo, sulfo, amino and	ratio of 9 to 1 (determined by using proton NMR) with Amax at 665
ether substituents.	nm. Attempts to convert the amides 2 or 3 into the corresponding
To obtain the compounds of the invention, a substituted or	imides by following the methods used in converting aromatic
unsubstituted chlorin or bacteriochlorin is reacted by known	anhydrides into imides, (e.g. such as heating with imidazole at
methods, as described in Kenner et al., J. Chem. Soc., Perkin	140°C) mainly gave decomposition products. Leaving the amide
Trans. I, 1973, 2517, to obtain a six membered anhydride ring	solution in dichloromethane or tetrahydrofuran at room
fused to the macrocyle. For example, to obtain a suitable	temperature for a week gave a mixture of purpurins with cyclic
bacteriochlorin, bacteriochlorophyll a is converted to	anhydride 1 (700 nm), cyclic imide 8 (705 nm) in minor amounts
bacteriopurpurin-a containing an anhydride ring before subjecting	and the starting material as a major product. By refluxing the
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imide analogue. Various attempts were then made to optimize the reaction mixture at various temperatures, slightly improved the any desired CH₂Cl₂ as a solvent again gave a mixture of cyclic imide 8 as minor product (12%) and afforded corresponding carbodiimides which are not stable and converted to corresponding isoimide analogues 6 and 7 imide 4 in Reaction of anhydride analog 1 as major product (85%), which were easily Reaction of purpurin amides mixture of 6-carboxyhexylamide 2 and γ -carboxy-hexylamide 3) (1:6) in 96% yield. This reaction is precisely the same reaction described in parent Application Serial No. 08/613,134, except that the useful end products were inadvertently mischaracterized Separation of the mixture gave pure isomers 6 and 7 with long Both Refluxing the isoimide (6 and 7) with various solvents alone or with K-10 clay gave mainly the starting material 1. Treatment of isoimides (either 6 or 7) with DBU/toluene at 60°C produced imide 8 in 60% yield. Interestingly, replacing DBU with stronger bases, such as methanolic KOH or NaOH at room temperature, gave This reaction was repeated several times using individual as the unstable carbodiimide analogs rather than isoimides. spectroscopy. the desired purpurin-imide in 85% overall yield (Amax 705nm). wavelength absorptions at Amax 696 and 690nm respectively. isoimide isomers (6 or 7), and produced the desired 0 U mass conditions, as summarized in Table 1. yield of purpurin-anhydride without formation isomers were characterized by proton NMR and amides 2 and 3 with K-10 clay using separated by column chromatography. DCC reaction with ø

Replacing DCC with 1,1'-thiocarbonyldiimidazole under similar reaction conditions gave purpurin-imide, but in a lower yield (Table 1). A proposed mechanism for the formation of the imide ring in accordance with the invention is shown in Figure 2. In brief, addition of the carboxylic acid to the carbodimide will give an O-acylisourea, an activated carboxylic acid derivative. It is now understood that these intermediate species are not stable and convert to corresponding isoimides and dicyclohexylurea. Intramolecular nucleophilic attack under basic conditions will generate cyclic imide. In tetrapyrrole chemistry, this is the first example of the formation of cyclic isoimides and imides from appropriate dicyclohexylcarbodiimide intermediate with methanolic XOH.

Related carbodiimide and isoimide analogs were prepared using bacteriopurpurin-a 12 as a substrate and converting it to the related imide derivative. Bacteriopurpurin-a 12 was isolated from *R*. Spheroides by following the methodology as described in U.S. Patent Application Serial No. 08/247,866 by reaction of bacteriochlorin with n propanol.

on reacting with dicyclohexylcarbodiimide is believed to produce t t Я Reaction of 12a with n-hexylamine gave the amide analogs 13 and 14, which (minor treatment convert the corresponding unstable carbodiimide derivatives 15 bacteriochlorophyll-a from R-speroides with n-propanol. which à compound 12a was obtained component) (major 16 and Another component)

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>80% yield without formation of purpurin-18 methyl ester

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respectively. corresponding isoimides with long wavelength absorption at $\lambda \max$ compounds with hydrophobic properties is in progress, e.g., (a) to increase/decrease the length of alkyl amides by opening the ç D) with t t replace position 2-(q) acids, (b) and t t position -7, ring introduce primary- or secondary alkyl ether groups at other carbodiimides, Û (000,000) the synthesis of a series of related anhydride ring with various amines, and amino various esters or aspartic acid analogs, 804 methylester group (at and Amax 18 dicyclohexylcarbodiimide with 796nm 17 (£=89,000) of the macrocycle. the currently, various replace

For a compound to be useful for PDT and IR imaging, it should have preferential accumulation in tumor. In an initial experiment, the uptake of bacteriopurpurin 18 in tumor vs. muscle was measured by in vivo reflection spectroscopy. From Figures 6A and 6B it can be seen that bacteriopurpurin 18 shows preferential accumulation of drug in tumor than muscles (8:1). Studies with other related compounds are currently in progress.

<u>Experimental</u>

Chemistry:

Commercially available compounds and reagents were purchased from Aldrich, ACROS Organics and Sigma. Mps were taken using a Fisher-Johns hot plate melting point apparatus and are uncorrected. NMR spectra were recorded at 300 MHz on a GE instrument using CDCl₃ as solvent. Electronic absorption spectra were recorded using a Genesis-5 spectrophotometer.

New and novel method for the preparation of purpurin-imide from isoimide via carbodiimide intermediate:

Was Ч. dichloromethane (100 ml) was treated with 1-hexylamine (2 ml, 2 Spectrophotometry was used to monitor disappearance of the peak ż solvent hexylamine 12% reaction mixture (220 these The was dissolved in dichloromethane (25 ml) and The solvent into The mechanism of the formation of these compounds is shown in Figs. 2 the preparation of purpurin-imide, the mixture of 6 and 7 (245 mg) was dissolved in min, reacted with dicyclohexylcarbodiimide (DCC) (400 mg, 1.75 mmol) was concentrated to 10 ml and left overnight in the refrigerator; à L L using and mg/10 by-product was removed mmol) 20 residue filtration. The filtrate was concentrated and separated ۲Û derivatives. (9 The structure of 5 were not stable. The The reaction mixture was stirred for 84 for t t KOH (0.5 0.34 crystallized from dichloromethane/hexane to give Ļ under a nitrogen atmosphere with stirring for 12 h. 700 nm and appearance of a new peak at 666 nm. the ч derivatives 2 (major) and 3 (minor) as a mixture temperature the ratio of , ęm was then removed under high vacuum; and For to be isoimide THF (50 ml), and a methanolic solution of The (200 The yield was 90% (245 mg). yields, respectively (total: 220 mg). н gel). 4 and room dicyclohexylurea formed as a individual isomers 4 and 5 (in ester preparative plates (silica intermediate carbodiimides stirred at were determined methyl Purpurin-18 water) was added. mmol) and compounds 0.34 and 4. mmol) , DE at

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and was monitored by spectrophotometry (appearance of a new peak The mixture was then diluted with dichloromethane The (100 ml) and washed with water (3 x 100 ml). The organic layer from For the syntheses of Evaporation of the solvent gave a residue which was chromatographed on a silica gel but were converted into the desired imides in one-pot without after 8 was carbodiimides and isoimide analogs were not isolated, other purpurin-imides 9-11 (see Table 2), the intermediate appropriate eluates were combined. The residue obtained dichloromethane/hexane, and the desired purpurin-imide column (elution with 2% acetone/dichloromethane). crystallized was dried over anhydrous sodium sulfate. in 85% yield (185 mg from 1). vas solvent further purification. the at 705 nm). evaporating obtained amides,

Isoimide derivative of Bacteriopurpurin-a propyl ester (17) and (18):

The the and the residue was crystallized from dichloromethane/hexane to obtained from R. spheroides was dissolved in dichloromethane (100 mixture reaction mixture was stirred at room temperature for 24h; peak at 813nm (due to starting material) and appearance of a new 0.05 mmol), The solvent was then removed under high vacuum, as spectrophotometry was used to monitor the disappearance of mmol). (major) The reaction 0.1 Bacteriopurpurin-a propyl ester **12a** (30 mg, give hexylamine derivatives 13 (minor) and 14 ml) and was treated with 1-hexylamine (0.2 ml, mixture in 10 and 90% yields respectively. peak at 786nm.

(30 mg, 0.05 mmol) was dissolved in dichloromethane (25 ml) and mg, 0.25 mmol) The solvent The filtrate was concentrated and separated into individual isomers 17 and 18 (in the ratio of 1 to 9) using to 10 ml and left overnight in the refrigerator; a by-product was removed by 72% overall). be с С found under a nitrogen atmosphere with stirring for 12 h. On the basis of NMR data, these compounds were preparative plates (silica gel). Yield: (28 mg, (50 (DCC) isoimide derivatives of bacteriopurpurin-a reacted with dicyclohexylcarbodiimide as formed dicyclohexylurea was concentrated filtration.

Spectroscopic Data:

Purpurin-18-N-hexylimide Methyl Ester (8): [Fisher's Nomenclature]

Mp. 221-223°C. UV/Vis: ($\lambda \max/nm$, ε): 705 (46,000); 647 (12,000); 549 (23,000); 510 (10,000); 483 (8,000); 417 (120,000). ¹H NMR (δ ppm, CDCl₃): 9.63 (s, β -meso H), 9.38 (s, α -meso H), 7.92 (dd, J 19.5, 12.8 Hz, 2a-H), 6.29 (dd, J 19.5 Hz, 2b-H), 6.18 (dd, J 12.8 Hz, 2b'-H), 5.37 (d, J 8.5 Hz, 7-H), 4.48 (t, N-hexylimide-a-CH₂), 4.38 (q, J 8.0 Hz, 8-H), 3.84 (s, 1-7H), 4.48 (t, N-hexylimide-a-CH₂), 4.38 (q, J 8.0 Hz, 8-H), 3.4 (s, 1-7H), 4.48 (t, N-hexylimide-a-CH₂), 3.56 (s, OMe), 3.34 (s, 1-7H), 2.66 (m, 7-1H), 2.65 (m, 7b-H), 2.51 (m, 7b'-H), 2.40 (m, 7a-H), 2.06 (m, 7a-H), 2.00 (m, N-hexylimide-b, C-CH₂CH₂), 1.74 (d, J 8.0 Hz, 8-Me), 1.65 (t, J 7.2 Hz, 4-b Me), 1.43 (m, N-hexylimide-d, e-CH₂CH₂), 0.46 (t, J 7.8 Hz, N-hexylimide-f-CH₃), - 0.08 and -0.17 (each br s, NH). m/z (LRMS): 661 (M+H).

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: (6)

Purpurin-18-15¹-hexylisoimide methyl ester (7)

Mp. 138-139°C. ¹H NMR (δ ppm, CDCl₃): 9.73 (s, β -meso H), 9.49 (s, α -meso H), 8.65 (s, δ -meso H), 7.92 (dd, J 19.4, 12.2 Hz, 2b'-H), 5.26 H), 6.31 (dd, J 19.4 Hz, 2b-H), 6.18 (dd, J 12.2 Hz, 2b'-H), 5.26 (d, J 8.5 Hz, 7-H), 4.57 (q, J 8.0 Hz, 8-H), 4.10 (m, hexyl isoimide-CH₂), 3.83 (s, 5-Me), 3.76 (q, J 7.5 Hz, 4a-CH₂), 3.58 (s, OMe), 3.46 (s, 1-Me), 3.28 (3-Me), 2.65 (m, 7b-H), 2.58-2.00 (m, 7b'-H; m, 7a-H; m, 7a'-H; m, hexyl isoimide-b,c-CH₂CH₂; 1.78 (d, J 8.0 Hz, 8-Me), 1.72 (t, J 7.2 Hz, 4-b Me), 1.61 (m, hexylamide-d, e-CH₂CH₂), 0.96 (t. J 7.8 Hz, hexyl isoimide f-CH₃), -0.61 and -0.88 (each br s, NH). m/z (LRMS): 661 (M + H).

Purpurin-18- 13¹-hexyl isoimide methyl ester (6)

Mp. 142-143°C. ¹H NMR (δ ppm, CDCl₃): 9.74 (s, β -meso H), 9.57 (s, α -meso H), 8.75 (s, δ -meso H), 7.94 (dd, J 19.2, 12.5 Hz, 2a-H), 6.33 (dd, J 19.2 Hz, 2b-H), 6.15 (dd, J 12.5 Hz, 2b'-H), 5.24 (d, J 8.5 Hz, 7-H), 4.52 (q, J 8.0 Hz, 8-H), 4.06 (t, hexyl isoimide-a-CH₂), 3.81 (s, 5-Me), 3.74 (q, J 7.5 Hz, 4a-CH₂), 3.56 (s, OMe), 3.42 (s, 1-Me), 3.24 (3-Me), 2.65 (m, 7b-H), 2.51-2.00 (m, 7b'-H; m, 7a-H; m, nexylisoimide-b,c-CH₂CH₂; m, dicyclohexylisourea-CH₂), 1.76 (d, J 8.0 Hz, 8-Me), 1.68 (t, J 7.2 Hz, 4-b Me), 1.58 (m, hexyl isoimide-d,e-CH₂CH₂), 0.98 (t, J 7.8 Hz, hexylisoimide-f-CH₃), -0.66 and -0.84 (each br s, NH).

Purpurin-18-N-hexylimide-7-N-aspartylamide Dimethyl Ester [Fisher's Nomenclature]

(43,200); 648 8.58 (s, ð-meso H), 7.90 (dd, J 19.8, 12.6 Hz, 2a-H), 6.98 (d, J 9.6, aspartate-NHCO), 6.32 (dd, J 19.8 Hz, 2b-H), 6.18 (dd, J aspartate-CH), 3.82 (s, 5-Me), 3.69 (s, OMe), 3.64 (q, J 7.5 Hz, ¹H NMR (δ ppm, CDCl_3): 9.60 (s, eta-meso H), 9.34 (s, lpha-meso H), ł Ē 2.06 (m, 7a'-H), 1.99 (m, N-hexylimide-b,c-CH₂CH₂), 1.75 (d, *J* 4a-CH₂), 3.61 (s, OMe), 3.36 (s, 1-Me), 3.16 (3-Me), 2.84 (m, 7b'-H), 2.46 (m, 7a-H), (11,000); 549 (21,000); 510 (9,200); 483 (7,800); 417 (112,000). 8.0 Hz, 8-Me), 1.66 (t, J 7.5 Hz, 4-b Me), 1.45 (m, N-hexylimided, e-CH₂CH₂), 0.96 (t, J 7.8 Hz, N-hexylimide-f-CH₃), -0.38 and Hz, 7-H), 4.46 (t, hexylisoimide-a-CH₂), 4.44 (g, J 8.0 Hz, 8-H), 4.38 705 : (3 0.11 (each br s, NH). m/z (LRMS): 791 (M+H) aspartate-CH₂), 2.64 (m, 7b-H), 2.51 (m, (λ max/nm, Hz, 2b'-H), 5.34 (d, J 8.5 uv/vis: Mp. 218-219°C. 12.6

Purpurin-18-N-hexylimide-7-N-Aspartylamide-di-tert-butyl Ester (10): Mp. 190-192°C. UV/Vis: (λ max/nm, ε): 705 (42,800); 648
(11,000); 549 (20,000); 510 (9,000); 483 (7,500); 417 (110,000).
¹H NMR (δ ppm, CDCl₃): 9.63 (s, β-meso H), 9.37 (s, α-meso H),
8.58 (s, δ-meso H), 7.86 (dd, J 19.4, 12.5 Hz, 2a-H), 6.21 (dd, J
9.6 Hz, aspartate-NHCO), 6.32 (dd, J 19.4 Hz, 2b-H), 6.21 (dd, J
12.5 Hz, 2b'-H), 5.33 (d, J 8.5 Hz, 7-H), 4.66 (q, J 8.0 Hz, 8-H), 4.41 (t,N-hexylimide-CH₂), 3.99 (m, aspartate-CH), 3.18 (3-Me),
H), 4.41 (t,N-hexylimide-CH₂), 3.36 (s, 1-Me), 3.18 (3-Me),

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2.76 (m, aspartate-CH ₂), 2.65 (m, 7b-H), 2.51 (m, 7b'-H), 2.4 (m, 7a-H), 2.06 (m, 7a'H), 1.92 (m, N-hexylimide-b,c-CH ₂ CH ₂) 1.68 (d, J 8.0 Hz, 8-Me), 1.66 (t, J 7.5 Hz, 4-b Me), 1.37 (s aspartate- ^t Bu), 1.34 (s, aspartate- ^t Bu), 1.14 (m, N-hexylimide	4 Q 	0 .	, 3.94 (t, 2H, 3.17 (s, 3H, 3- + 8a-CH ₂ + 7b'-
d,e-CH ₂ CH ₂), 0.95 (t, J 7.8 Hz, N-hexylimide-f-CH ₃), -0.38 and 0.11 (each br s, NH). Mass: m/z (HRMS): requires f C ₅₁ H ₆₇ N ₆ O ₇ : 875.5071. Found 875.5016.	- ц	H), 2.14 (m, H, 17a-H), 1.98 (m, H, 17a'-H), 1.81, 1.73 (each d, 3H, J = 8.0, 18-Me, 7-Me), 1.11 (t, 3H, J = 7.8, 3-b Me), 0.83 (t, 3H, J = 8.2, CH ₂ CH ₂ CH ₃), -0.30 and -0.67 (each br s, 2H, 21, 222-MV	1.73 (each d, 3-b Me), 0.83 br s, 2H, 21,
Purpurin-18-N-glycylimide-tert-Butyl Ester-7-aspartylamide-di- tert-butyl Ester (11): [Fisher's Nomenclature] Mp. 138-139°C. UV/Vis: (λ max/nm, ε): 705 (41,300); 649 (10,000): 549 (19,000): 510 (8,700); 483 (7,300): 417 (105,000).	1	iopurpurin-a-15 ¹ -N-hexylisoimide (17): aturej: Àmax: 795 (67,000); 537 (24,500); 410	[Fisher's (50,400); 363
Layouov, με μαρω, με μαινόνι με με με μαινόνι με μαινόνι με μαινόνι με μαινόνι μα μαινόνι μα μα μα μα μα μα μα ¹ Η NMR (δ ppm, CDCl ₃): 9.61 (s, β-meso H), 9.35 (s, α-meso H) 8.56 (s, δ-meso H), 7.88 (dd, J 19.5, 12.6 Hz, 2a-H), 6.65 (d, 9.5 Hz, aspartate-NHCO), 6.26 (dd, J 19.5 Hz, 2b-H), 6.16 (dd,	• • • •	600). NMR (CDCl ₃ , ð ppm): 9.21 (s, 1H, 5-H), 1), 8.68 (s, 1H, 20-H), 5.35 (m, 2H, NHCO + 17 3-H, 18-H), 4.11 (m, 3H, 8-H + hexylamide-a-C	(s, 4.32 3.91
12.5 Hz, 2b'-H), 5.27 (d, J 8.5 Hz, 7-H), 5.18 (q, glycine-CH ₂) 4.68 (q, J 7.5 Hz, 8-H), 4.38 (m, aspartate-CH), 3.38 (s, 5-Me) 3.64 (q, J 7.5 Hz, 4a-CH ₂), 3.35 (s, 1-Me), 3.16 (3-Me), 2.79 (m aspartate-CH ₂), 2.66 (m, 7b-H), 2.54 (m, 7b'-H), 2.46 (m, 7a-H) 2.01 (m, 7a'-H), 1.73 (d, J 7.5 Hz, 8-Me), 1.67 (t, J 7.5 Hz, 4-) Me) 1.58 (s, division-t and 1.28 (s, div)si))))))))))))))		$C_{2}C_{12}D_{1}$, J. OY (S, JH, 12-ME), 3.58 (S, JH, 2-W) $3-ME$), 2.62 (m, H, 17b-H), 2.44 (m, 5H, $CH_{2}CH_{2}C$) H), 2.14 (m, 6H, 17a-H + hexylisoimide-b, $c-CH$, 1.84 (each d, 3H, $J = 8.0$, 18-Me, 7-Me), lamide- d , $e-CH_{2}$, 1.11 (t, 3H, $J = 7.8$, 3-b Me), lamide- $f-CH_{3}$), 0.88 (t, 3H, $J = 8.2$, $CH_{2}CH_{2}CH_{3}$).	le), 3.19 (s, H ₃ + Ba-CH ₂ + 2 + 17a'-H), 1.60 (m, 4H, 0.97 (t, 3H,
Mey, 1.04 (2, 9170105 - 24), 1.04 (5, 35Partare - 24), 1.04 (5 aspartate- ^t Bu), 0.10 and -0.04 (each br s, NH). m/2 (LRMS) 905. (M+H). Bacteriopurpurin-a 17-propyl Ester (12a): [Fisher's Nomenclature		1.03 (each br s, 2H, 21, 23-NH). Mass: LRMS:708(M+H) Bacteriopurpurin-a-13 ¹ -N-hexyl isoimide (18) Nomenclature	
UV/Vis (CH ₂ Cl ₂ , Àmax, nm): 813 (56,000); 543 (32,000); 401 (48,000); 363 (102,000). NMR (CDCl ₃ , ð ppm): 9.21 (s, 1H, 5- H) 8.79 (s, 1H, 10-H), 8.62 (s, 1 H, 20-H), 5.14 (d, 1H, J=8.0, 17	co ~ 1	UV/Vis (À max/nm, ɛ): 804 (82,800); 539 (33,200); 409 (59,4 360 (94,000). NMR (CDCl ₃ , ð ppm): ď H: 9.38 (s, 1H, 5-H), (s, 1H, 10-H), 8.73 (s, 1H, 20-H), 5.46 (m, 1H, NHCO), 5.1E	(59,400); 5-H), 8.88 5.18 (d,
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1H, J = 8.0, 17-H), 4.34 (m, 2H, 3-H, 18-H), 4.17 (m, 1H, 8-H), 3.91 (m, 2H, hexylisoimide-a-CH₂), 4.06 (t, 2H, CO₂CH₂), 3.68 (s, 3H, 12-Me), 3.59 (s, 3H, 2-Me), 3.19 (s, 3H, 3-Me), 2.73 (m, H, 17b-H), 2.42 (m, 5H, $CH_2CH_3 + 8a-CH_2 + 7b'-H$), 2.14 (m, H, 17a-H), 2.08 (m, 5H, hexylamide- $b, c-CH_2+17a'-H$), 2.01, 1.93 (each d, 3H, J=8.0, 18-Me, 7-Me), 1.57 (m, 4H, hexylisoimide- $d, e-CH_2$), 1.12 (t, 3H, J = 7.8, 3-b Me), 0.96 (t, 3H, hexylamide- $f-CH_3$), 0.87 (t, 3H, J = 8.2, $CH_2CH_2CH_3$), -0.86 and -1.13 (each br s, 2H, 21, 23-NH). Mass: LRMS:708(M+1)

Biological Studies:

Determination of drug uptake:

The absorption spectrum of a compound in living tissue can The be recorded using an instrument and technique which we have The experiment measures the light which scatters Xenon arc lamp and passes through a grating monochromator to a 90 tissue an experimental mouse tumor) and the light is collected by a second fiber placed in contact with the tissue at a fixed The noninvasive character of this measurement makes data collection possible at a number of time points after the i.v. injection of an experimental The light signal is detected by a photodiode. The photo current (Z H pressure light absorbing compound (e.g., a potential photosensitizer). 06) quartz fiber. the through the tissue. The light originates in a high converted into a voltage, amplified by a tuned in contact with distance (3 to 5 mm) from the first fiber. Hz chopper and then into a 400 μ m diameter distal end of this fiber is placed developed. (e.g., ,s

amplifier and synchronously detected. The chopping at 90 Hz and synchronous detection makes examining spectra under normal room lighting possible.

the these experiments, the mice were first anesthetized The optical vitro absorption spectrum. The drug was then administered by tail vein second spectrum contains a component caused by the presence of the drug in the This in vivo drug absorption spectrum is best displayed double beam absorption spectrophotometer. The pre-injection i.v. (typically a cuvette and solvents) and the post-injection data as plus by taking the ratio of the post-injection spectrum to the preas a sample these two spectra is certainly not influenced by the wavelength dependence of the As a safeguard to day or hour to hour drift in the total light output of the lamp, both spectra (pre- and post-injection) are normalized by dividing the signal strength at a wavelength where ц Ц the reference beam injection spectrum. This ratio offers the same advantages expected longest wavelength of the experimental drug's inset beam power as a function of wavelength was recorded before injection of the sensitizer. The monochromator is using either Pentobarbital or Ketamine Xylazine i.p. mouse data can be thought of as the reference light signal which characterizes the instrument. The the sample beam containing everything in the injection and the light signal recorded. The ratio of the drug absorption is negligible. the experimental drug. against day For tumor.

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Photosensitizing Efficacy: Part I - In vivo Efficacy of Bacteriochlorins 17 and 18 using SMT-F Tumor Model

The new photosensitizer was screened in a mouse/tumor model system. A model system consisted of observing the size reduction of the SMT-F tumor, a fast growing spontaneous mouse mammary tumor subline, transplanted subcutaneously to male or female DBA/2 HA-DD mice. The tumor line was maintained in vivo via serial transplantation in the same mouse strain. DBA/2 Ha-DD mice are readily available and were obtained locally.

mm cube) were transplanted with a 18 gauge trocar from a donor This technique provides for relatively uniform tumor size and allows location of the tumor in the right the drug, the mouse was weeks) and weight (approximately 20 g), small pieces of tumor (1-When tumor reached 4-5 mm in diameter, the animals were injected group overgrown and Three auxiliary region of the animal within each experimental group. Only animals with single tumors were chosen for experiments. (approximately chosen from the surrounding the tumor was removed with electric clippers. Prior to irradiation, the fur appropriate age twenty four hours after injecting with the potential photosensitizer placed in a custom-made aluminum holder the were both tumor to recipient mouse. described above. When mice 占 2

Irradiation Conditions:

Standard light dose was 75 mW/cm² for 30 min for a total incident dose of 135 J/cm^2 from a tunable dye laser tuned to the maximum red absorption peak. Spectra Physics 2040, a quartz

fiber fitted with a microlens was interfaced to the dye laser deliver a uniform field of light. Laser output was measured with a power meter. Further studies at various light doses and treatment conditions are currently in progress.

Experimental Procedure:

ഹ and ad 80 days after of non-responsive tumor require Following light exposure, the mice were kept in groups of water tumor cage and supplied with pelleted food and tap both overlying surrounding skin was monitored daily for of libitum. Tumor size and gross appearance early sacrifice of those animals. photoillumination unless growth per

The photosensitizer was dissolved in known quantity of Tween 80 (Aldrich) surfactant and diluted by a factor of 10 with saline solution to produce a final Tween 80 concentration of 10-20%. The solution was then filtered through a syringe filter. The concentration of the solution was determined on the basis of the extinction coefficient value of the photosensitizer at the longest wavelength absorption. Absorption spectra were obtained using a Spectronic Genesis5 spectrophotometer.

Before injecting the drug into mice, the purity of the compounds was ascertained by analytical HPLC using Spectra Physics HPLC, connected with C8 reverse phase column, eluted with methanol/water by adjusting the pH to 7.0 using phosphate buffer.

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The time for growth

by interpolation of

was then estimated

to the tumor to 400 mm³

Tumor

response was recorded on the basis of the number of animals which were found to be tumor free. Appropriate controls were carried

the times just before and after 400 $extsf{mm}^3$ was reached.

out with tumor-bearing mice which received no treatment at all,

or received light or photosensitizer only.

using the formula $v=(lw^2)/2$, where l is the longest axis of the

tumor and w is the axis perpendicular to 1.

Alkylimide derivatives of bacteriochlorins:

Procedures similar to preparation of N-hexylimide derivatives were followed except that various other alkyl amines were substituted for hexylamine. The resulting compounds were tested for in vivo antitumor activity.

4-6 mm diameter tumors (DBA/2 mice transplanted with SMT-F tumors) were exposed to 75 mW/cm² for 30 minutes to deliver light (135 J/cm^2) from a tunable dye laser tuned to a maximum red absorption peak. The tumors were non-palpable and five mice were used per group.

The results are in Table 4. The results indicate a large suppressive effect for isomers where the extended alkyl group is closest to the amino acid group. Part II - In vivo efficacy of bacteriochlorins 17 and 18 using Radiation Induced Fibrosarcoma (RIF) Tumor Model. A mixture of bacteriochlorins 17 and 18 was also evaluated for *in vivo* PDT efficacy using another model (RIF tumor moded) routinely used in our laboratory. In brief, six mice per group with appropriate tumor size (4-5 mm) were chosen for the experiment. As shown in Table 1, the animals were treated at variable doses of light and drug. Beginning 24h after PDT, and at least every other day thereafter, tumors were measured in orthogonal diameters with an electronic caliper (ultra-Cal Mark III; Fred V. Fowler Co., Boston MA). Each measurement was utomatically recorded, where the tumor volume, V, was calculated 28

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Table 2. Yields of purpurin-imides

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Table 1. Formation of purpurin-18 with cyclic anhydride and imide rings

at various reaction conditions

Merinod No.	Condiaons		Yield (%)	(<u>v</u> 2
		imide 4	anhydride 1	anhydride 1 รเลกเกรู materials (5 & 6)
LT.	THF. reiluxing for 4h.	ý	70	25
2. tair	lmidazole. I≟0ºC. Ih	Decomp	Decomposition products	
3. K-10	K-10 Clay. CH2Cl2, 24h	10-12	80-85	0
U T	CH2Cl3, 10 days	15-20	20-25	50
5. DCC with:				
л. Ч	K-10 Clay, RT	00	00	100
	OBU. toluene. rcflux 2h	60 C	00	004
	KOH/MeOH RT 10 min	50	c	
		0	þ	>
6. TCD with:				
4. DF	a. DBU.RT	0	0	100
b. DB	DBU. tolune. reflux 2h	10	50	•
20 B 7 U 7	. DBU. THF 60°C	30	0	60
RT S		01	0	06
DCC: Dicyclohex	DCC: Dicyclohexyicarbodiimide: DBU, 1.8-Diazabicyclo(5.4.0)undec-7-ene: RT, Room	1.8-Diazabic;	clo(5.4.0)undec	.ī ~ne: RT. Room
		IGAZOIC.		

_ S	% yield	85
HN NH NH O HN NH O OH3 OH3 OH3 OH3 OH3 OH3 OH3 OH3 OH3 OH3	RI	n-hexyl
U I U U U	ĸ	OMe
	oN ba	ĺ

% yield	85	89	87	78
R	n-hexyl	n-hexyl	a-hexyl	<i>ter</i> t-butyl Gly
ĸ	OMe	Asp-di-methyl ester	Asp-di- <i>ter</i> r- butyl ester	Asp-di-rerr- buryl ester
Compound No.	60	6	10	11

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Table 3. PDT Efficacy in Mice Transplanted with Radiation Induced Fibrosarcoma (RIF) Tumor with a Mixture of Bacteriochlorins 17 and 18

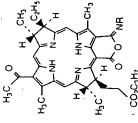
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TABLE 4

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CO2C3H

80\$

100%

60%

80%

r

ັບ ກິ

80\$ 40%

100% 60%

100%

24 24 24 24

75 0 8 75 30

0.20 0.20 0.25 0.25

80% 100\$ 80%

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Tumor Response days

Timer after Injection hrs

Light Dose Rate mW/cm²

Drug Dose μmol/kg

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(MAJOR (804 NM) CO₂C₃H₇

18. R = (CH₂)₅CH₃20. R = (CH₂)₅CH₃22. R = (CH₂)₂CH₃22. R = (CH₂)₂CH₃24. R = (CH₂)₉CH₃

 $\begin{array}{rcl} 17. \ R &= \ (CH_2)_5 CH_3 \\ 19. \ R &= \ (CH_2)_5 CH_3 \\ 21. \ R &= \ (CH_2)_2 CH_3 \\ 21. \ R &= \ (CH_2)_2 CH_3 \\ 23. \ R &= \ (CH_3)_5 CH_3 \end{array}$

MINOR (795 NM)

Comparative in vivo Antitumor Activity of Bacteriochlorins	Tumor response (d) ^{†,*}	REGROWTH ON DAY 15	NO RESPONSE	REGROWTH ON DAY 4
Comparative in vivo Antitumor Activity of Bacteriochlorins	Time(h) betw. injection and light treatment	======================================		24
rative in vivo	in vivo absorption (λ max)	804	804	804
Compa	Dose (µmol/kg)	0.47	0.47	0.47
	Compound	======================================	20	2.2

9 100 5 804 0.47 Mixture of 23 and 24

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⁷ 4-5 mm diameter turnors were exposed to 75 MW/cm² for 30 min to deliver 135 J/cm² light from a tunable dye laser tuned to the maximum red absorption peak. d = days.

Non-palpable tumors.

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WHAT IS CLAIMED IS:	where R is selected from the group consisting of OMe and Asp-di-
1. A method for the manufacture of an imide derivative of	onsi
purpurin comprising:	hexyl and tert-butyl Gly.
reacting hexylamine with a chlorin or bacteriochlorin having	
a macrocycle with a six membered anhydride ring fused thereto,	ther reacted with an alkali
said macrocycle containing a and b rings which may be saturated	
or unsaturated at ${ extsf{R}_4}$ to ${ extsf{R}_{11}}$ positions of the rings and which ${ extsf{R}_4}$	
and ${f R}_{11}$ positions may contain at least one group selected from	5. An imide of purpurin manufactured in accordance with the mothod of others.
the group consisting of hydrogen, hydroxy, formal, substituted	mernod of Claim 1.
and unsubstituted alkyl, alkoxy, alkenyl, aryl and aryloxy	6. An imide of purpurin manufactured in accordance with the
wherein carbon containing groups may be substituted with a	
substituent selected from carboxyl, hydroxy, phosphoro, carboxy,	
halo, sulfo, amino and ether, to obtain a purpurin derivative;	 A reaction product comprising an imide of purpurin
and reacting the purpurin derivative with a carbodiimide to	manufactured in accordance with the method of Claim 3.
obtain the imide derivative of purpurin.	8. A reaction product comprising an imide of purnurin
2. The method of Claim 1, wherein the carbodiimide is	od of Claim 4.
dicycloberylcarbodiimide.	9. A compound of the formula:
3. The method of Claim 1, wherein the imide derivative is	
further reacted with an alkali metal hydroxide to obtain a	
purpurin imide of the formula:	R ₁₀ R ₂ R ₂ R ₂ R ₂
;	
	H H
H HN CH3	H ₃ C ['] H ₁₂
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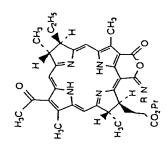
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where z is = 0 or = NR_{14} ; R_1 is an amino acid group, a polyamine group, a polyether group or OR_{13} where R_{13} is alkyl; R_{14} is alkyl, substituted alkyl, a polyamine group, or an amino acid group; R_4 through R_{11} are -H, -OH, alkyl, alkylene, $-OR_{16}$, where R_{16} is H, alkyl or aryl, or a carbonyl containing group, provided that; R_4 may be taken together with R_5 to form =0; R_6 may be taken together with R_1 to form =0; R_6 may be taken together with R_{11} to form =0; R_1 may R_4 and R_7 may be taken together with R_{11} to form =0; and R_9 to form =0; R_{10} may be taken together with R_{11} to form =0; and R_4 and R_7 may together form a chemical bond and R_8 and R_{11} may together form a chemical bond; and R_{12} is hydrogen or lower together form a chemical bond; and R_{12} is hydrogen or lower alkyl; provided that if one z is = 0, the other z is = NR_{14} .

10. The compound of Claim 9 wherein R_{11} and R_{12} are -CH₃. 11. The compound of Claim 9 wherein R_2 is an allyl group.

12. The compound of Claim 9 wherein R_3 is an allyl group.

13. The compound of Claim 9 having the formula:



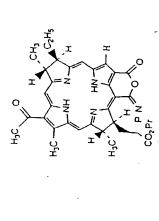
where R is normal alkyl of 2 through 12 carbon atoms.

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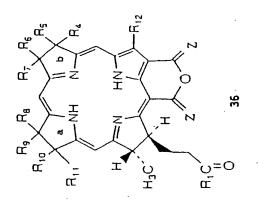
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14. The compound of Claim 9 having the formula:



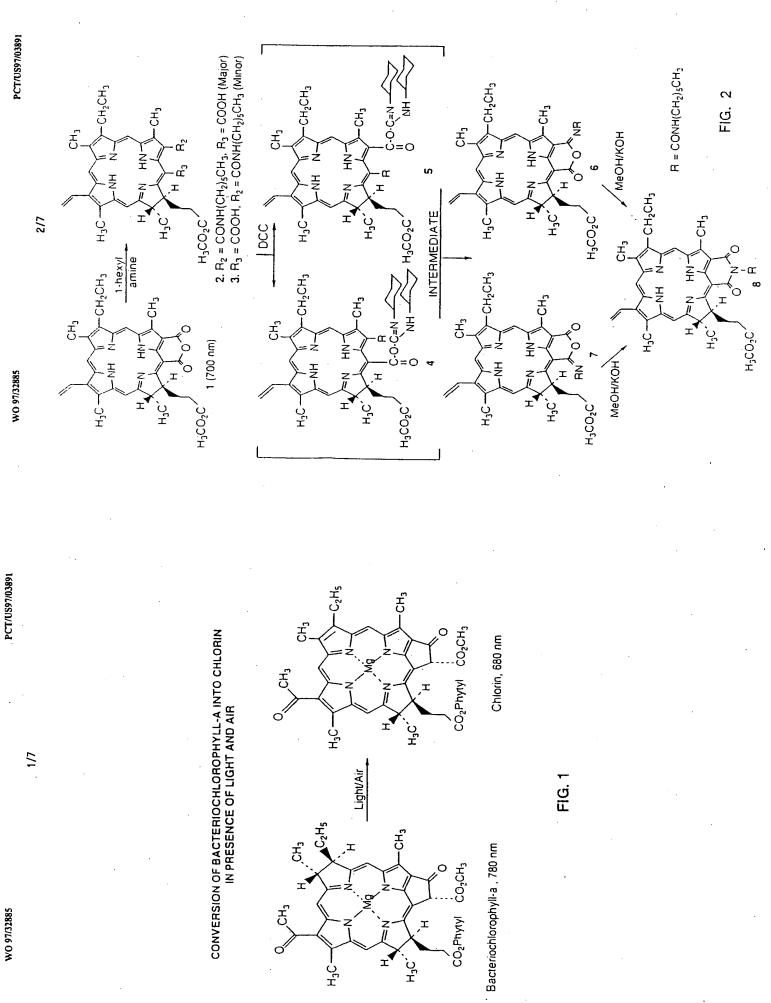
where R is normal alkyl of 2 through 12 carbon atoms. 15. The compound of Claim 10 wherein R₂ is O=COR₁₅. 16. The compound of Claim 10 wherein R₁ is H₃CO₂C-. 17. The compound of Claim 15 wherein R₁ is H₃CO₂C-. 18. The compound of Claim 16 wherein R₂ is H₃CO₂C-. 18. The compound of Claim 16 wherein R₂ is H₃CO₂C-. 19. A method for the manufacture of an isoimide derivative of purpurin comprising reacting a purpurin of the formula:



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id group, a polyamine group,	ther	26. A method for the manufacture of purpurin imide comprising
	uted	
alkyl, a polyamine group, or an amino acid group; R ₄ through R ₁₁ are -HOH. alkyl, alkyleneOR,,, where R., is H, alkyl or	R ₁₁ l or	
, or a carbonyl containing group, provided that, R		27. A method for the manufacture of purpurin imide comprising
taken together with $ extsf{R}_{ extsf{S}}$ to form =0; $ extsf{R}_{ extsf{G}}$ may be taken together with	with	reacting the purpurin derivative from Claim 19 with alkali metal
R_7 to form =0; R_8 may be taken together with R_9 to form =0;	=0; R ₁₀	hydroxide.
may be taken together with ${ m R_{11}}$ to form =0; and ${ m R_{4}}$ and ${ m R_{7}}$	may	28. A method for the manufacture of an imide derivative of
together form a chemical bond and ${ m R}_{ m B}$ and ${ m R}_{ m 11}$ may together fo	form a	
smical bond; and R_{12} is hydrogen or lower alkyl; provided	that	reacting hexylamine with a chlorin or bacteriochlorin having
one z is =0, the other z is =NR $_{14}$, with 1-hexylamine to	open	a macrocycle with a six membered anhydrid ring fused thereto,
nydride ring followed by r	e to	said macrocycle containing a and b rings which may be saturated
obtain the isolmide derivative of purpurin.		or unsaturated at \mathbb{R}_4 to \mathbb{R}_{11} positions and which \mathbb{R}_4 to \mathbb{R}_{11}
20. A method for the manufacture of purpurin imide comprising	sing	positions may contain at least one group selected from the group
reacting the compound of Claim 9 with alkali metal hydroxide		consisting of hydrogen, hydroxy, formal, substituted and
		unsubstituted alkyl, alkoxy, alkenyl, aryl and aryloxy, wherein
anufacture of purpurin imide	SIDG	carbon containing groups may be substituted with a substituent
reacting the compound of Claim 10 with an alkali metal hydroxide	11de.	selected from carbonyl, hydroxy, phosphoro, carboxy, halo, sulfo,
22. A method for the manufacture of purpurin imide comprising	sing	amino and ether, to obtain a purpurin derivative; and
reacting the compound of Claim 15 with an alkali metal hydroxide	tide.	reacting the obtained purpurin derivative with a
23. A method for the manufacture of purpurin imide comprising	sing	carbodiimide to obtain the imide derivative of purpurin.
reacting the compound of Claim 16 with an alkali metal hydroxide	tide.	
24. A method for the manufacture of purpurin imide comprising	sing	
reacting the compound of Claim 17 with an alkali metal hydroxide	cide.	
25. A method for the manufacture of purpurin imide comprising	sing	
reacting the compound of Claim 18 with an alkali metal hydroxide	cide.	· ·
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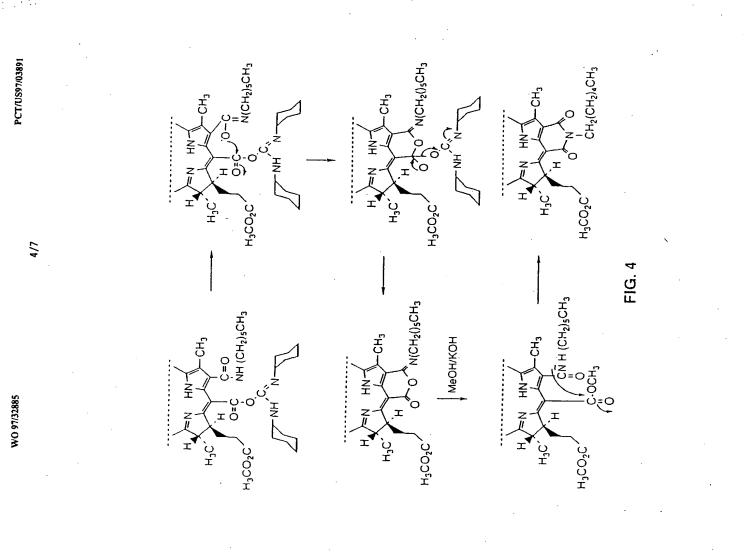
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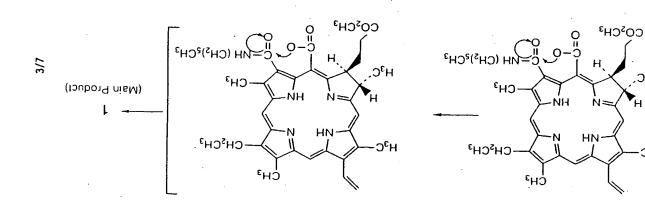
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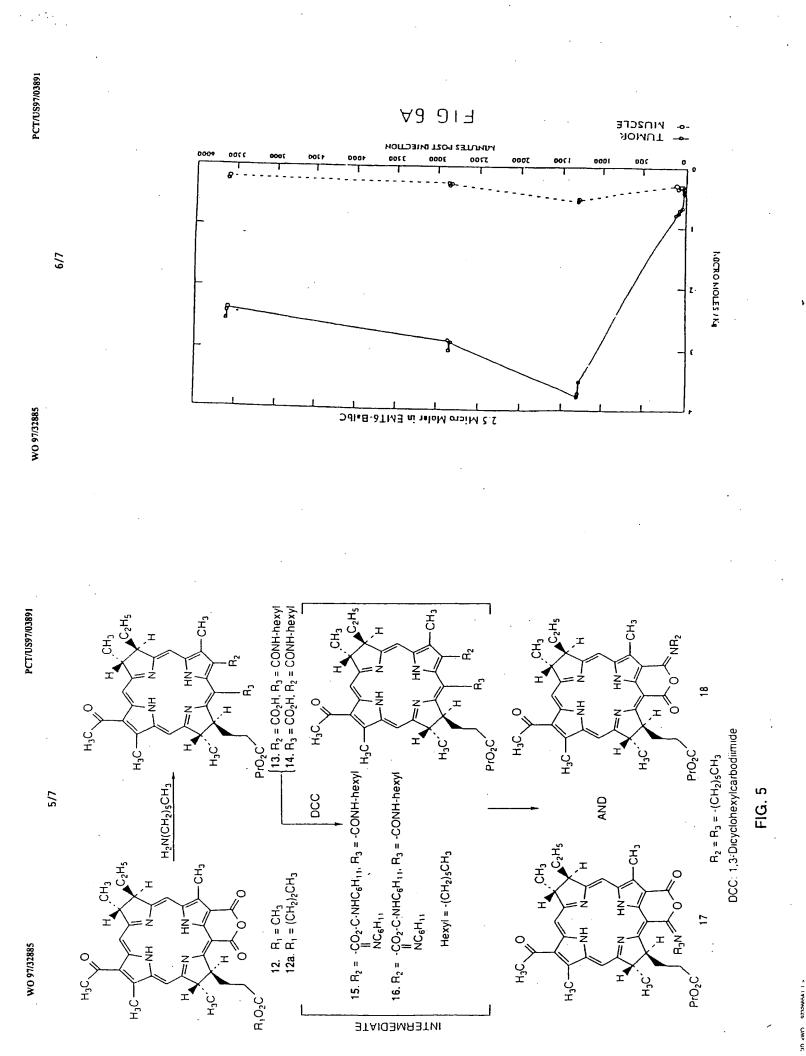
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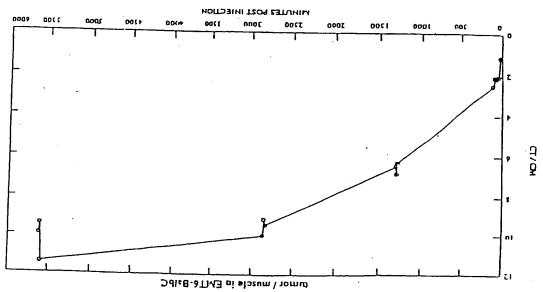
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A. CLA IPC(6) US CL According	 CLASSIFICATION OF SUBJECT MATTER IPC(6) : C07D 487/22 US CL : 540/145,472,474 According to International Patent Classification (IPC) or to both national classification and IPC 	national classification and IPC		r
B. FIE	FIELDS SEARCHED			<u>г т</u>
Minimum o U.S. :	Minimum documentation searched (classification system followed by classification symbols) U.S. : 540/145,472,474	d hy classification symbols)		
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20 ن	DOCUMENTS CONSIDERED TO BE RELEVANT			
Category*	Citation of document, with indication, where appropriate, of the relevant passages	propriate, of the relevant passages	Relevant to claim h	
X, P	US 5,591,847 A (PANDEY et al) entire document.	(PANDEY et al) 07 JANUARY 1997 see	1.28	
_×	LEE et al. Use of the Chlorophyll Derivate, Purpurin-18, for Syntheses of Sensitizers for Use in Photodynamic Therapy., Jorn. Chem. Soc, Perkin Trans I. October 1993, pages 2369- 2372, especially page 2369 and formulae on page 2370.	Derivate, Purpurin-18, for h Photodynamic Therapy ctober 1993, pages 2369- ormulae on page 2370.	1-28	
≻	FIESER AND FIESER, Reagents for Organic 1,1967, page 233. N.Y. John Wiley & Sons.	r Organic Synthesis, Vol y & Sons.	1-4 and 20-28.	
Eurth	Further documents are listed in the continuation of $Box\ C$.	See patent family annex.		
	Special cavegories of cated documents: document defining the graceral state of the art which in not considered	*T have document published after the international filing date or priority date and point conflict with the application but clack to understand the primeriphe or theory underlying the investion	metional filing date or priority thon but cited to understand the muton	
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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT) [51] International Patent Classification 6 [51] International Patent Classification 6	1 20	 (30) Priority Data: (30) Priority Data: 08/613.134 8 March 1996 (08.03.96) US With international search report. Not furnished 6 March 1997 (06.03.97) US With international search report. Date of publication of the amended claims: (71) Applicant: HEALTH RESEARCH, INC. [US/US]; Roswell 	Park Mermorial Institute Division, 666 Elm Street, Buffalo, NY 14263 (US). (72) Inventors: PANDEY, Ravindra, K.: 75 Lemay Court, Williamsville, NY 14221 (US), KOZYREV, Andrei, N.: U75 Cambridge Boulevard #1, Amherat, NY 14226 (US). DOUGHERTY. Thomas. J.: 2306 W. Oakfield, Grand Island, NY 14072 (US).	(74) Agent: DUNN, Michael, L.; Dunn & Associates, P.O. Box 96, Newfanc, NY 14108 (US).	(54) TIUE: SYNTHESIS OF ISOIMIDE OF CHLORINS AND BACTERIOCHLORINS AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF CANCER	ing compounds naving utility as light absorb- ing compounds, especially in the area of pho- lodynamic therapy. Such compounds have for- mula (1), where 2 is = 0 or NR14; R14 is alkyl or substituted alkyl. R1 is an amino acid group, a polyamine group, a bolyamine group, a polyamine group of OR1, a bolyamine group of R1, are H. OH.	why, for a carbonyl containing group, provided that: Ramy be keen together with R ₂ to form -0: R ₃ may be taken together with R ₃ to form -0: R ₃ may be taken together with R ₁ to form -0: R ₃ may be taken together with R ₁ to form -0: R ₃ may be taken together with R ₁ to form -0: and R ₄ and R ₃ may together form a chem. (cal bond; and R ₁₁ is hydrogen or lower ability: provided that if one z is 0, the other z is -0; R ₁₁ to the other z is -0; R ₁₂ to the other z is 0. the other z is -0; R ₁₂ to the other z is 0. the other z is	νυ=0 Φ	

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and Asp-di-		ng of n-		derivative is	a cyclic		with the		with the			purpurin		purpurin													
nsisting of OMe		selected from the group consisting		1, wherein the imide	cali metal hydroxide to obtain		manufactured in accordance	-	manufactured in accordance			an product comprising an imide of accordance with the method of Claim 3.		comprising an imide of	with the method of Claim 4.	ula:		հ, Բե Բ _Հ Բե		NH N-)	z o z		40	AMENDED SHEET (ARTICLE 19)
R is selected		methyl ester and $ extsf{R}_1$ is se	hexyl and tert-butyl Gly.	4. The method of Claim	further reacted with an alkali	imide.	5. An imide of purpurin	method of Claim 1.	6. An imide of purpurin	method of Claim 2.		a reaction in		8. A reaction product	manufactured in accordance	9. A compound of the formula:		C		L H	Ţ				0=ر Ē		AMENDI
AMENDED CLAIMS	on 9 Sentember 1007 (00 00 07)	our 5 september 1777 (02.02.23 and 25 cancelled; inal claims 12, 15, 16, 18, 22, 23 and 25 cancelled; tanged (6 pages)	ure of an imide derivative of		reacting hexylamine with a chlorin or bacteriochlorin having	d anhydride ring fused thereto,	which may be saturat	o K ₁₁ positions of the rings and which R ₄ contain at least one group selected from	n, hydroxy, formyl, substituted	alkoxy, alkenyl, aryl and aryloxy	ps may be substituted with a	selected from carboxyl, hydroxy, phosphoro, carboxy,	to obtain a purpurin derivative;	vative with a carbodiimide to	of purpurin.	wherein the carbodiimide is		wherein the imide derivative is	alkali metal hydroxide to obtain a		CH ₃	6		HIN CH3		- œ	TICLE 19)
A MENDED (Ireceived by the International Bureau on 9 Semember 1907 (09:09 07)	original claims 1. 2, 9, 11, 17 and 19 amended: original claims 12, 15, 16, 18, 22, 23 and 25 cancelled. remaining claims unchanged (6 pages)	1. A method for the manufacture	purpurin comprising:	reacting hexylamine with a c	a macrocycle with a six membered	said macrocycle containing a and	or unsaturated at \aleph_4 to \aleph_{11} positions of and \aleph_{11} positions may contain at least o	the group consisting of hydrogen,	and unsubstituted alkyl, alkox	wherein carbon containing groups	substituent selected from carboxy	halo, sulfo, amino and ether, tc	and reacting the purpurin derivative with	obtain the imide derivative of pu	2. The method of Claim 1,	dicyclohexylcarbodiimide.	3. The method of Claim 1, wh	further reacted with an alkali	purpurin imide of the formula:	ſ	H ₅ C			T		AMENDED SHEET (ARTICLE 19)

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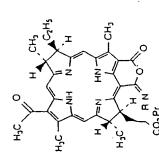
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where z is = 0 or = NR_{14} ; R_{1} is an amino acid group, a polyamine or an amino acid R14 IS substituted with carboxy, halo, sulfo, amino and ether substituents, provided that; R_4 may be taken together with group; R₄ through R₁₁ are -H, -OH, alkyl, alkoxy, alkenyl, $^{
m R_5}$ to form =0; $^{
m R_6}$ may be taken together with $^{
m R_7}$ to form =0; $^{
m R_8}$ alkylene, aryl, or aryloxy, or a carbonyl containing group, taken together with R_{11} to form =0; and R_4 and R_7 may together form a and R_{12} is hydrogen or lower alkyl; provided that if one z is = chemical bond and $extsf{R}_8$ and $extsf{R}_{11}$ may together form a chemical bond; may be taken together with $extsf{R}_{ extsf{0}}$ to form =0; $extsf{R}_{ extsf{10}}$ may be group, a polyether group or OR_{13} where R_{13} is alkyl; alkyl, substituted alkyl, a polyamine group, may be groups carbonyl, hydroxy, phosphoro, wherein carbon containing 0, the other z is = NR_{14} .

10. The compound of Claim 9 wherein R_{11} and R_{12} are $-CH_3$.

The compound of Claim 9 wherein R_9 is -COCH $_3$. .11

The compound of Claim 9 having the formula: 13.



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AMENDED SHEET (ARTICLE 19)

where R is normal alkyl of 2 through 12 carbon atoms.

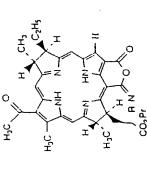
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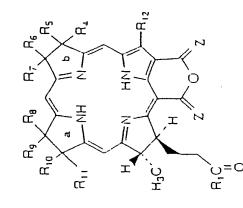
The compound of Claim 9 having the formula:

14.



where R is normal alkyl of 2 through 12 carbon atoms.

The compound of Claim 9 wherein R_1 is $H_3CO_2C^-$ 17. 19. A method for the manufacture of an isoimide derivati purpurin comprising reacting a purpurin of the formula:



AMENDED SHEET (ARTICLE 19)

wherein z is =0 or NR_{14} ; R_1 is an amino acid group, a polyamine л. alkyl, or substituted alkyl, a polyamine group, or an amino acid substituted with hydroxy, phosphoro, carboxy, halo, sulfo, amino and ether substituents provided that; ${ t R}_4$ may be taken together with R4 through R₁₁ are -H, -OH, alkyl, alkoxy, alkenyl, alkylene, aryl, or aryloxy, or a carbonyl containing group, $^{
m R_5}$ to form =0; $m R_6$ may be taken together with $m R_7$ to form =0; $m R_8$ be taken together with ${
m R}_{1\,1}$ to form =0; and ${
m R}_4$ and ${
m R}_7$ may together form a chemical bond and ${
m R}_8$ and ${
m R}_{11}$ may together form a chemical bond; the other z is =NR $_{14}$, with 1-hexylamine to open the anhydride and R_{12} is hydrogen or lower alkyl; provided that if one z is =0, a carbodiimide to obtain the R_{14} a polyether group or OR_{13} where R_{13} is alkyl; may be taken together with R_9 to form =0; R_{10} may wherein carbon containing groups may be reaction with isoimide derivative of purpurin. ring followed by carbonyl, group; group,

20. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 9 with alkali metal hydroxide.

21. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 10 with an alkali metal hydroxide. 24. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 17 with an alkali metal hydroxide.

26. A method for the manufacture of purpurin imide comprising reacting the purpurin derivative from Claim 19 with alkali metal hydroxide. 27. A method for the manufacture of purpurin imide comprising reacting the purpurin derivative from Claim 19 with alkali metal hydroxide.

4.3 AMENDED SHEET (ARTICLE 19)

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28. A method for the manufacture of an imide derivative of purpurin comprising:

R11 reacting hexylamine with a chlorin or bacteriochlorin having consisting of hydrogen, hydroxy, formal, substituted and unsubstituted alkyl, alkoxy, alkenyl, aryl and aryloxy, wherein positions may contain at least one group selected from the group saturated a macrocycle with a six membered anhydrid ring fused thereto, selected from carbonyl, hydroxy, phosphoro, carboxy, halo, sulfo, carbon containing groups may be substituted with a substituen ç which R4 macrocycle containing a and b rings which may be amino and ether, to obtain a purpurin derivative; and unsaturated at \mathbb{R}_4 to \mathbb{R}_{11} positions and said 5 U

reacting the obtained purpurin derivative with a carbodiimide to obtain the imide derivative of purpurin.

AMENDED SHEET (ARTICLE 19)

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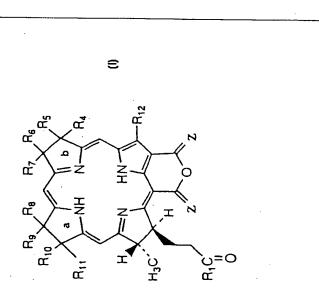
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(51) International Patent Classification ⁶ :		(11) International Publication Number: WO 97/32885
C07D 487722	A1	2 Septemt
(21) International Application Number: PCT/US	168E0/L6	PCT/US97/03891 (81) Designated States: CA, JP. MX, European patent (AT, BE,
(22) International Filing Date: 7 March 1997 (07.03.97)	(79.60.70	CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(30) Priority Data: (30) Priority Data: 08.6(13,134 Not furnished 6 March 1997 (06.03.97)	SU SU	Published With international search report. With amended claims.
(71) Applicant: HEALTH RESEARCH, INC. [US/US]; Roswell Park Memorial Institute Division, 666 Elm Street, Buffalo, NY 14263 (US).	Roswell Buffalo,	Date of publication of the amended claims: 6 November 1997 (06.11.97)
(72) Inventors: PANDEY, Ravindra, K.; 75 Lemay Court, Williamsville, NY 14221 (US), KOZYREV, Andrei, N.; 175 Cambridge Boulevard #1, Amherst, NY 14226 (US), DOUGHERTY, Thomas, J.; 2306 W. Oakfield, Grand Island, NY 14072 (US).	y Court, Idrei, N.; 26 (US). I Grand	
(74) Agent: DUNN, Michael, L.; Dunn & Associates, P.O. Box 96, Newfane, NY 14108 (US).	Box 96,	
(54) Title: SYNTHESIS OF ISOIMIDE OF CHLORINS TREATMENT OF CANCER	AND B.	(54) Trule: SYNTHESIS OF ISOIMIDE OF CHLORINS AND BACTERIOCHLORINS AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF CANCER

(57) Abstract

aryl, or a carbonyl containing group, provided thai: R_4 may be taken together with R_3 to form -0: R_6 may be taken together with R_1 to form -0: R_6 may be taken together with R_1 to form -0: R_0 may be taken together with R_1 to form -0: R_0 may be taken together with R_1 to form -0: and R_4 and R_7 may together form a chem-tical bond and R_8 and R_1 may together form a chemical bond; and R_1 is hydrogen or lower alkyl; provided that if one z is 0, the other z is $-NR_1$. Compounds having utility as light absorbing compounds, especially in the area of photodynamic therapy. Such compounds have formula (1), where z is = 0 or NR14; R14 is alkyl a polyamine group, a polyether group or OR13 where R13 is alkyl; R4 through R11 are -H, -OH, alkyl, alkylene, -OR16 where R16 is H, alkyl or or substituted alkyl. RI is an amino acid group.



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SYNTHESIS OF ISOIMIDE OF CHLORINS AND BACTERIOCHLORINS AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF CANCER

Background of the Invention

This invention relates to treatment and diagnosis of cancer through the use of IR imaging and photodynamic therapy employing porphyrin related compounds and more particularly certain analogs of chlorins and bacteriochlorins. Photosensitizers are chemicals which kill cells and/or fluoresce when activated by light of a specific wavelength. Most malignant and some premalignant tissues retain these photochemically active substances in higher concentrations and for longer durations than surrounding normal tissues. The retention time is not dependent on whether or not the cells are synthesizing DNA or undergoing cell growth or nutrient uptake.

Light sensitive drugs, lasers and fiber optic probes have been combined in a procedure known as photodynamic therapy (PDT). PDT has emerged as one of the most promising strategies in cancer treatment (including cancer detection). PDT is being increasingly used where chemotherapy, surgery and high energy increasingly used where chemotherapy, surgery and high energy irradiation have failed. In this new therapy, patients are given intravenous injections of a photodynamic drug that accumulates in cancer cells in much higher concentrations than in normal cells. The photodynamic (photosensitizing) drug is then activated to

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through fiber optics. People who are too old or too frail to kill the cancer cells by a laser beam directed to cancer cells tolerate the stress of major surgery, chemotherapy or high energy radiation may be helped by photodynamic therapy, which often requires just local anesthesia and does not necessitate hospital cancer therapy that has many additional advantages, e.g. it can be performed any number of times on a single patient, it is not contraindicated with other cancer therapies and it allows selective treatment of malignant tissues due to preferential retention of dye in cancer P early for cells and it has already been established that superficial malignancy, PDT may be curative. admission. PDT is an important form of

The these two ideas (photodegradation of tissue and localization in tumors) came together successfully, when Diamond demonstrated that a porphyrin could preferentially degrade tumor implants in a Nielsen, S.; Jaenicke, R., Lancet, 1972, 1175). This result was In 1900, (Rabb, C., Z. Biol., 1900, 39, 1423) reported the lethal effects of a combination of acridin orange dye and ordinary light therapeutic use of photosensitizers when he used eosin and white localization of administered porphyrins in tumor tissue was recognized in the 1940's. It was not until 1972, however, that rat (Diamond, I.; McDonagh, A.F.; Wilson, C.B.; Granelli, S.G.; of an Photosensitizers have been recognized for almost a century. first confirmed and extended by Dougherty, T.J.; Grindey, G.B.; Fiel, the administered porphyrin in man was observed in 1913. The phototoxic effect reported von Tappeneir to treat skin tumors. In 1903, on Paramecium. light

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R.; Weishaupt, K.R.; Boyle, D.G.; J. Natl. Cancer Inst., 1975, 55, 115.

avoid malignant tumors For detection early stage small tumors, the porphyrin-containing tumor cells The porphyrins then emit a strong fluorescence, which contrasts with the much The and irradiating the tumor area with a wavelength of light which Mittleman, A.; Cancer Res., 1976, 38, 3628). PDT techniques normal tissue, allowing for detection. For the treatment of cancer, photodynamic therapy (PDT) consists of injecting the patient with a photoactive dye surrounding environment by producing single oxygen and oxygen radicals (Dougherty, T.J.; J.H.; Goldfarb, A.; Weishaupt, K.R.; Boyle, D.; depend strongly on how well the compound used preferentially concentrates within the tumor cell. Skin photosensitivity is the only known side effect of PDT with certain porphyrin type photosensitizers. Because skin retains these chemicals in enough tumor. patients must to produce toxins which kill the used for the treatment and detection of cancer. and surrounding tissues are exposed to light. The higher concentration of porphyrins in quantities to produce surface reactions, porphyrin dyes become toxic to the weaker fluorescence from the exposure to sunlight. activates the dye Kaufman, is. ų

The distribution of porphyrin drugs in the body compared with tumor cells is still under investigation. The distribution varies with cell type and porphyrin derivative. It is thought that once the photosensitizer is injected intravenously, some of

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the the drug escapes the blood stream and moves into the interstitial The porphyrin binds to the cellular membrane and slowly Each porphyrin, then, rapidly Fluorescence shows within cells membrane and binds to hydrophobic regions inside the cell. L1210 porphyrin-treated leukemia plasma diffuses into the cell cytoplasm. localization around the intracellular vesicles. microscopy of fluid.

Photofrin®, a hematoporphyrin derivative (Dougherty, T.J.; used all over the world for the treatment of a variety of solid Boyle, D.G.; Weishaupt, K.R., "Photodynamic Therapy - Clinical New York, 1983, p. 3) is the only photosensitizer currently being mixing Hpd thus produced consists of a variety of porphyrins. When and Drug Advances, Porphyrin Photosensitization," Plenum Press, is separated into its two main fractions by gel filtration Sephadex LH-20, the higher molecular weight portion, called T.J.; . . M 1983, 160, 3). body oligomers linked with ether, and possibly carbon-carbon linkages R.K.; Siegel, M.M.; Tsao, R.; McReynolds, J.M.; acid, followed by hydrolysis and precipitation under acidic conditions. (Lipson, higher R.L.; Baldes, E.J.; Olsen, A.M., J. Natl. Cancer Inst., 1961, 26, Boyle, D.G.; Weishaupt, K.R.; Henderson, B.; Potter, of hematoporphyrin with glacial acetic acid and sulfuric Photofrin $({f B})$, is a more efficient PDT agent (Dougherty, The main components of Photofrin® are dimers and prepared by The recommended human dosage of Photofrin $(\ensuremath{\mathbb{B}}\)$ is 1-2 mg/kg al Lipson et Bellnier, D.A.; Wityk, K.E., Adv. Exp. Biol. Med., Hematoporphyrin derivative (Hpd) is partially described by Vas This method (Pandey, tumors. weight. with Нpd

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(Pandey, R.K.;

This was attributed to the increased

Bellnier, D.A.; Smith, K.M.; Dougherty, T.J., Photochem.

Photobiol., 1991, 53, 65).

pheophorbide-a, pyropheophorbide- and chlorin₆.

hydrophobicity of the hexyl group and is consistent with studies

done by Evenson on porphyrins with varying polarities

J.F.; Sommer, S.; Riminfton, C.; Moan, J., Br. J. Cancer, 1987,

(Evenson,

Dougherty, T.J., Biomed. and Environ. Mass Spectrometry, 1990, 19, 405)

å non-toxic, selectively taken up and/or retained in malignant nm), and photochemically efficient. Although Photofrin ${
m (B)}$ has been approved for commercialization in Canada, Europe and the United States, it lacks rapid clearance from tissues, is a complex mixture of oligomers, and has the disadvantage that its absorbance at 630 nm porphyrin 0 F a photosensitizer to be clinically useful, it must improvement New penetrating light (>600 photosensitizers are thus needed for the is not optimized for tissue penetration. photodynamic therapy for cancer treatment. γd tissues, activated For

There is a need for more efficient, chemically pure, less phototoxic, and better localizing porphyrins. Important prior Proc. SPIE, 1989, 1065, 104. The aspartyl derivatives of chlorin e_6 , monoaspartyl chlorin e_6 and diaspartyl chlorin e_6 , were found Cancer to be responsible for the efficiency of tissue clearance. In pheophorbide, pyropheophorbide and chlorin e_f series, certain alkyl ether derivatives including 2-(1hexyloxyethyl)2-des vinyl derivatives were found to be excellent art porphyrin and chlorin derivatives have been reviewed by Pandey, R.K.; Majchrzycki, D.F.; Smith, K.M.; Dougherty, T.J., group methyl to be effective photosensitizers in vitro (Roberts, W.G.; Shaiu, Inst., 1988, 80, 330). With these compounds, the aspartyl F.Y.; Nelson, J.S.; Smith, K.M., Roberts, M.W., J. Natl. compounds, photosensitizers compared with parent was noted

Commun., 1986, 1213, have previously shown that chlorins, on Chang, C.K., Sotiroiu, C.; Wu, W,, J. Chem. Soc., Chem. 55, 483)

bacteriochlorin system. This methodology has been extended in reacting with osmium tetroxide can be converted to vic dihydroxy preparation at a series of vic -dihydroxy and keto-bacteriochlorins (Pandey, R.K.; & Med. Chem. Lett., 1992, 2, 491). It has also been reported that the regiospecificity of pyrrole subunits in osmium tetroxide oxidation is affected significantly by the presence of electron bacteriochlorins, prepared from mesochlorin e $_{\mathsf{6}}$ trimethylester and pyropheophorbide-a methylester, have strong absorption in the red vivo photosensitizing activity (Kessel, D.; Smith, K.M.; Pandey, R.K.; These stable Shiau, F.Y.; Sumlin, A.B.; Dougherty, T.J.; Smith, K.M., Bioorg. 58, Shaiu, F.Y.; Henderson, B., Photochem. Photobiol., 1993, region (730 to 750 nm), but, did not show any significant in withdrawing substituents in the macrocycle(5a). λq pheophorbide-a and chlorin e₆ series, the 200)

Photobiol., 1988, 48, 579 showed that purpurin-18 2, which has Photochem. Hoober, J.K.; Sery, T.W.; Yamamoto, Y.,

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 terrong sheeption at 70 nm wight be a umeful patcemention. the past been period by cyclining clutring patcembon siture conventional and the part been period by cyclining clutring clutr		
compound 8) have in the past been prepared by cyclizing chil osensitizers, defining cyclized reaction mixture communication under etcompounds where strong diazomethane). The resulting cyclized reaction mixture communications becompounds photoactivate more of products in addition to the cyclic amine, espect photoactivate more of number of products in addition. Further such compounds come naturally significant subsequent purification. Further such compounds in a number of products in addition of the bravings photoactivate mot only inefficient because of low yield, but requires in the optimal hydrophylic-lipophylic balance. I reported as vivo (Beems, past, Brief Description of the bravings figure 1 is a schematic equation showing the synthetic r tetriochlorins in Figure 2 is a schematic equation showing the synthetic r tetriochlorins in Figure 2 is a schematic equation showing the synthetic r in results in figure 2 is a schematic equation showing the synthetic r tetriochlorin in Figure 2 is a schematic equation showing the synthetic r in resolution in Figure 2 is a schematic equation showing the synthetic r concluding the compound 1. Figure 4 is a schematic equation showing the synthetic r intermediates to compound 1. Figure 4 is a schematic equation showing the synthetic r educing the cyclic songound 1. Figure 6 is a schematic equation showing the synthetic r risochlorin in results in the schematic equation showing the synthetic r induction the compound 1. Figure 6 is a schematic equation showing the synthetic r risochlorin in results of a schematic equation showing the synthetic r risochlorin in results a schematic equation showing the synthetic r results in the schematic equation showing the synthetic r results of the invention in results of the invention. Figure 6 is a schematic equation showing the synthetic r results of the invention in results of the invention. Figure 6 is a schematic equation showing the synthetic r results of the schematic equation of the invention. Figure 6 is a curve showing the ratio o	a useful photosensitizer	
P6 6-Wiexylamide-1-methyl seter 3A (compound 3 reacted nrial useful unresstud diazomethame). The resulting cycliced reaction mixture com where strong diazomethame). The resulting cycliced reaction mixture com where strong number of products in addition to the cyclic amine, especiated photoactivate number of products in addition to the cyclic amine, especiated sues (Pandey, animet of products in addition to the cyclic amine, especiated sues (Pandey, animit aubeequent purificant unreacted succed sues (Pandey, significant subsequent purification. Further such compounds one only inefficient because of low yield, but requision networks one only inefficient because of low yield, but requision succed one only inefficient because of low yield, but requision succed one only inefficient because of low yield, but requision succed one only inefficient because of low yield, but requision succed one head optimal hydrophylic-lipophylic bilance. Distriction figure 1 is a schematic equation showing the synthetic r terriochlorins intermediates to compound 8. it thus the intermediates to compound 1. it thus the if gure 2 is a schematic equation showing	photodynamic therapy (PDT).	compound 8) have in the past been prepared by cyclizing chlorin-
ntial useful a number of pro where strong unreacted start photoactivate not only inef ssues (Pandey, gherty, T.J.; ome naturally not had optimal some naturally not had optimal some naturally not had optimal n reported as Figure 1 is vivo (Beems, Figure 2 is vivo (Beems, Figure 2 is rintermediates to figure 5 is ricchlorin in Figure 5 is to cyclic isoimide bact ricchlorin in Figure 6 is to meet the figure 6 is f	long wavelength absorbing photosensi	6-N-hexylamide-7-methyl ester 3A (compound 3 reacted
where strong a number of products in addition to photoactivate ourreacted starting material 3A. The photoactivate ourreacted starting material 3A. The ssues (Pandey, ssues (Pandey, style in the file of the construction of gherty, T.J.; Some naturally reported as vivo (Beems, A.B.; Smeets, A.B.;	been proposed as potential	
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<pre>qperty, T.J.; significant subsequent purification. Further such not had optimal hydrophylic-lipophylic balance. iome naturally n reported as vivo (Beems,</pre>	-	only inefficient because of low yield, but
norm naturally not had optimal hydrophylic-lipophylic balance. n reported as Brief Description of the Drawing the backer, vivo (Beems, Figure 1 is a schematic equation showing the backer, A.B.; Smeets, Figure 2 is a schematic equation showing the backer, A.B.; Smeets, Figure 2 is a schematic equation showing the backer, A.B.; Smeets, Figure 2 is a schematic equation showing the backer, n); thus the ro compound 6. n); thus the ro compound 8. n); thus the ro cyclic anhydride, compound 1. n); thus the ro cyclic isoimides and cyclic imide. no chromophore Figure 5 is a schematic equation showing the to cyclic imide. ricochlorin in ro cyclic isoimides and cyclic imide. m chromophore Figure 6 is a schematic equation showing the to isoimide bacteriochlorins 17 and 18 of the inverse to isoimide bacteriochlorins 17 and 18 of the inverse to isoimide bacteriochlorins 17 and 18 of the inverse backeric dent. nave both an referential accumulation of compound 18 in tumors reluci agent. difficult and upounds (e.g. e.g.	Shiau, F.Y.; Isaac, M.; Ramaprasad, S.; Dougherty,	Further such
Interported as Brief Description of the Drawings vivo (Beems, Figure 1 is a schematic equation showing the A.B.; Smeets, Figure 1 is a schematic equation showing the A.B.; Smeets, Figure 2 is a schematic equation showing the 1987, 46, Figure 2 is a schematic equation showing the 1987, 46, Figure 2 is a schematic equation showing the 1987, 46, Figure 2 is a schematic equation showing the Figure 3 is a schematic equation showing the intermediates to compound 1. Figure 4 is a schematic equation showing the to cyclic isolnides and cyclic imide. Figure 6 is a schematic equation showing the to cyclic isolnides and cyclic imide. Figure 6 is a schematic equation showing the to cyclic isolnide bacceriochlorins 17 and 18 of the inve Figure 6 is a curve showing the ratio of tu tu Isolande bacceriochlorins 17 and 18 of the inve Have both an Figure 6 is a curve showing the ratio of tu Figure 6 is a curve showing the ratio of tu eutic agent. Isolandificult and accumulation as represented in Figure 6A.	Some na	not had optimal hydrophylic-lipophylic balance.
<pre>vivo (Beems, vivo (Beems, A.B.; Smeets, bacteriochlorophyll-a to chlorin. figure 2 is a schematic equation showing the ch results in ch results in ch results in ch results in ch results in intermediates to compound 8. figure 3 is a schematic equation showing the to cyclic anhydride, compound 1. Figure 4 is a schematic equation showing the to cyclic isoimides and cyclic imide. figure 5 is a schematic equation showing the to cyclic isoimides and cyclic imide. figure 6A is a reflection spectroscopy have both an the both an to meet the to isoimide bacteriochlorins 17 and 18 in tumors figure 6B is a curve showing the ratio of tu difficult and mpounds (e.g.</pre>	occurring bacteriochlorins, have previously been reported as	<u>Brief Description of the Drawings</u>
 A.B.; Smeets, bacteriochlorophyll-a to chlorin. A.B.; Smeets, Pigure 2 is a schematic equation showing the to compounds 6 and 7 of the invention and intermediates to compound 8. b); thus the to compound 8. c) rist a schematic equation showing the to cyclic anhydride, compound 1. Figure 3 is a schematic equation showing the to cyclic indice, compound 1. Figure 4 is a schematic equation showing the to cyclic isoimides and cyclic indice. riochlorin in w chromophore reducing the to cyclic isoimides and cyclic indice. reducing the to cyclic isoimide bacteriochlorins 17 and 18 in tumors for the to meet the to both an to both an the both an to both an to both an to both an to meet the to isoimide bacteriochlorins 17 and 18 in tumors figure 6. figure 6. is a curve showing the ratio of tu action for the entic agent. 		Figure 1 is a schematic equation showing the
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<pre>ch results in intermediates to compound 8. Figure 3 is a schematic equation showing the synthetic rou ns are lost. Figure 4 is a schematic equation showing the synthetic rou riochlorin in Figure 4 is a schematic equation showing the synthetic rou w chromophore Figure 5 is a schematic equation showing the synthetic rou reducing the Figure 5 is a schematic equation showing the synthetic rou reducing the Figure 6 is a reflection spectroscopy curve showi have both an Figure 6 is a curve showing the ratio of tumors over muscle. Figure 6B is a curve showing the ratio of tumor over muscle figure 6B is a curve showing the ratio of tumor over muscle. figure 6B is a curve showing the ratio of tumor over muscle. figure 6B is a curve showing the ratio of tumor over muscle. figure 6B is a curve showing the ratio of tumor over muscle. figure 6B is a curve showing the ratio of tumor over muscle. figure 6B is a curve showing the ratio of tumor over muscle. fifticult and mpounds (e.g. Rounds (e.g.</pre>		compounds 6 and 7 of the invention and
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educing the Figure 5 is to isoimide bacte trivatives of Figure 6A have both an preferential accu to meet the Figure 6B is eutic agent. accumulation as r difficult and mpounds (e.g.		cyclic isoimides and cyclic imide.
to isoimide bacte Figure 6A have both an to meet the figure 6B is reutic agent. difficult and mounds (e.g.	outside the laser window, thus reducing	5 is
Rerivatives ofFigure 6A is a reflection spectroscopy curvehave both anpreferential accumulation of compound 18 in tumors over mto meet theFigure 6B is a curve showing the ratio of tumor overeutic agent.accumulation as represented in Figure 6A.difficult andaccumulation as represented in Figure 6A.mpounds (e.g.8	photodynamic efficiency.	isoimide bacte
have both an preferential accumulation of compound 18 in tumors over mutuated meet the ratio of tumor over eutic agent. Brigure 6B is a curve showing the ratio of tumor over difficult and accumulation as represented in Figure 6A. Moounds (e.g.	has been found that certain cyclic amide derivatives	
to meet the Figure 6B is a curve showing the ratio of tumor over eutic agent. accumulation as represented in Figure 6A. difficult and mpounds (e.g.	both	accumulation of compound 18 in tumors over m
eutic agent. accumulation as represented in Figure 6A. difficult and mpounds (e.g.	and the requisite stability to meet	tumor over
difficult and mpounds (e.g. 8		6A.
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Brief Description of the Invention

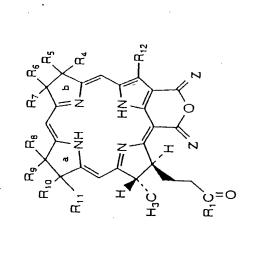
In accordance with the present invention, there are provided new chlorin and bacteriochlorin derivatives having utility as fluorescent and photosensitizing compounds. Such compounds may be excited by microwaves, ultrasound, and visible or infrared radiation.

All of such novel compounds described herein may be used in traditional areas where compounds having such properties have utility. The compounds, may, for example, be incorporated into a substance such as a plastic product, excited with ultrasound, microwaves or visible light followed by using known methods for detecting emitted radiation to image the product for the purpose of detecting voids or other flaws in the product.

Certain of such compounds have special utility as photosensitizers in the area of photodynamic therapy for the detection and treatment of tumors. In accordance with the invention, to make PDT more applicable, there is a need of long wavelength absorbing photosensitizers such as stable bacteriochlorins which have the ability to localize in high concentration at the tumor site.

Furthermore there is a need for an efficient and cost effective method for preparing such photosensitizers.

In accordance with the invention, a compound is therefore provided which comprises a chemical of the formula:



= NR14; R1 is an amino acid group, a polyamine group, a polyether group or OR_{13} where R_{13} is alkyl; $^{
m R}$ 14 is alkyl, or substituted alkyl, a polyamine group, or an amino acid group; ${
m R}_4$ through ${
m R}_{11}$ are -H, -OH, alkyl, alkylene, $^{-OR}_{16}$, where R_{16} is H, alkyl or aryl, or a carbonyl containing to form =0; R_6 may be taken together with R_7 to form =0; R_8 may be taken to form =0; and R_4 and R_7 may together form a chemical bond and together with R_9 to form =0; R_{10} may be taken together with R_{11} $^{
m R_{g}}$ and $^{
m R_{ll}}$ may together form a chemical bond; and $^{
m R_{l2}}$ is hydrogen ... other z group, provided that; ${
m R}_4$ may be taken together with ${
m R}_{
m S}$ the , 0= or lower alkyl; provided that if one z is 0 01 4 wherein z is =NR₁₄.

The invention further includes a method for using the above compound as an intermediate for the preparation of additional

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long wave length stable photosensitizers and a method for its preparation.

Detailed Description of the Invention

The invention permits more flexibility in the preparation of porphyrin-type compounds than was previously possible. Intermediate compounds may be provided with a number of substituents on the a and b rings and variable substituents at \mathbb{R}_1 .

The a and b rings may be saturated or unsaturated at the R_{4} - R_{11} positions or may contain hydrogen, hydroxy, formal or substituted and unsubstituted alkyl, alkoxy, alkenyl, aryl and aryloxy groups. The alkyl, alkoxy, alkenyl, aryl and aryloxy groups usually contain 1 through 8 carbon atoms and more commonly contain 1 through 3 carbon atoms. A limited number, i.e., as many as 2, of such carbon containing groups may be long chain carbon containing groups atoms.

The carbon containing groups may be substituted with carbonyl, hydroxy, phosphoro, carboxy, halo, sulfo, amino and ether substituents. To obtain the compounds of the invention, a substituted or unsubstituted chlorin or bacteriochlorin is reacted by known methods, as described in Kenner et al., J. Chem. Soc., Perkin Trans. I, 1973, 2517, to obtain a six membered anhydride ring fused to the macrocyle. For example, to obtain a suitable bacteriochlorin, bacteriochlorophyll a is converted to bacteriopurpurin-a containing an anhydride ring before subjecting

it to the subsequent reactions described herein. The anhydride is then reacted with 1, hexyl amine to open the anhydride ring, e.g., as shown in Figure 2 to obtain 6 carboxylhexyl amide 2 and γ -carboxy-hexylamide 3. The 6 carboxyhexylamide and the γ -carboxyhexylamide are then the R₁-R₁₂ reacted separately or together with a carbodiimide to form a porphyrin diimide which is unstable and immediately converts to carbodfimide is dicyloberylcarbodiimide (DCC), which results in preferred 4 where an isoimid, the compound of the invention. 2 in Figure substituents may vary as described herein. 2 and ە ţ compounds similar

The invention may be described in more detail by reference to the following specific embodiment.

665 imides by following the methods used in converting aromatic purpurin-18 methyl ester 1 was used as the starting material. As expected, reaction of 1 (Amax 700 nm) with 1-hexylamine gave the corresponding amides in 95% yield as a mixture of 2 and 3 in the corresponding anhydrides into imides, (e.g. such as heating with imidazole at E001 temperature for a week gave a mixture of purpurins with cyclic anhydride 1 (700 nm), cyclic imide B (705 nm) in minor amounts By refluxing the Leaving the amide Initially, in order to establish the reaction conditions, ratio of 9 to 1 (determined by using proton NMR) with Amax at at tetrahydrofuran nm. Attempts to convert the amides 2 or 3 into the and the starting material as a major product. 140°C) mainly gave decomposition products. solution in dichloromethane or

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imide analogue. Various attempts were then made to optimize the amides 2 and 3 with K-10 clay using $extsf{CH}_2 extsf{Cl}_2$ as a solvent again and any desired 7 with long 4 in reaction mixture at various temperatures, slightly improved the Reaction of easily Reaction of purpurin amides with DCC afforded corresponding carbodiimides which are not stable and converted to corresponding isoimide analogues 6 and 7 (1:6) in 96% yield. This reaction is precisely the same reaction that the useful end products were inadvertently mischaracterized Both Refluxing the isoimide (6 and 7) with various solvents alone or isoimides (either 6 or 7) with DBU/toluene at 60°C produced imide Interestingly, replacing DBU with stronger bases, such as methanolic KOH or NaOH at room temperature, gave This reaction was repeated several times using individual γ-carboxy-hexylamide 3) 08/613,134, except as the unstable carbodiimide analogs rather than isoimides. Treatment of >80% yield without formation of purpurin-18 methyl ester 1. mass spectroscopy. the desired purpurin-imide in 85% overall yield (Amax 705nm). product (12%) imide wavelength absorptions at Amax 696 and 690nm respectively. were or 7), and produced the desired which with K-10 clay gave mainly the starting material 1. purpurin-anhydride without formation of Separation of the mixture gave pure isomers 6 and Ŀ. in Table gave a mixture of cyclic imide 8 as minor product (85%), described in parent Application Serial No. proton NMR and of 6-carboxyhexylamide 2 and reaction conditions, as summarized column chromatography. as major characterized by isoimide isomers (6 anhydride analog 1 in 60% yield. isomers were separated by (a mixture of yield

Replacing DCC with 1,1'-thiocarbonyldiimidazole under similar reaction conditions gave purpurin-imide, but in a lower yield (Table 1).

the imide ring in now understood that these intermediate species are not stable and with give an 0-acylisourea, an activated carboxylic acid derivative. It is Intramolecular nucleophilic attack under basic conditions will the imides In brief, convert to corresponding isoimides and dicyclohexylurea. is appropriate dicyclohexylcarbodiimide intermediate and generate cyclic imide. In tetrapyrrole chemistry, this addition of the carboxylic acid to the carbodiimide will shown in Figure 2. isoimides proposed mechanism for the formation of cyclic first example of the formation of accordance with the invention is methanolic KOH from

Related carbodiimide and isoimide analogs were prepared using bacteriopurpurin-a 12 as a substrate and converting it to the related imide derivative. Bacteriopurpurin-a 12 was isolated from *R. Spheroides* by following the methodology as described in U.S. Patent Application Serial No. 08/247,866 by reaction of bacteriochlorin with n propanol.

on reacting with dicyclohexylcarbodiimide is believed to produce f Reaction 14, which corresponding unstable carbodiimide derivatives 15 (minor t t convert treatment with n-propanol. of 12m with n-hexylamine gave the amide analogs 13 and which à obtained component) bacteriochlorophyll-a from R-speroides was (major compound 128 16 and Another component) the

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various hydrophobic properties is in progress, e.g., (a) to increase/decrease the length of alkyl amides by opening the t t with ţ corresponding isoimides with long wavelength absorption at $\lambda \max$ Currently, the synthesis of a series of related compounds with introduce primary- or secondary alkyl ether groups at position 2-(E=109,000) respectively. to replace anhydride ring with various amines, and amino acids, (b) (q â dicyclohexylcarbodiimide with other carbodiimides, and replace the methylester group (at position -7, ring () () or aspartic acid analogs, and Amax 18 804 (800,000) of the macrocycle. various esters 17 796nm

For a compound to be useful for PDT and IR imaging, it should have preferential accumulation in tumor. In an initial experiment, the uptake of bacteriopurpurin 18 in tumor vs. muscle was measured by in vivo reflection spectroscopy. From Figures 6A and 6B it can be seen that bacteriopurpurin 18 shows preferential accumulation of drug in tumor than muscles (8:1). Studies with other related compounds are currently in progress.

<u>Experimental</u>

Chemistry:

Commercially available compounds and reagents were purchased from Aldrich, ACROS Organics and Sigma. Mps were taken using a Fisher-Johns hot plate melting point apparatus and are uncorrected. NMR spectra were recorded at 300 MHz on a GE instrument using CDCl₃ as solvent. Electronic absorption spectra were recorded using a Genesis-5 spectrophotometer.

New and novel method for the preparation of purpurin-imide from isoimide via carbodiimide intermediate:

Ľ. dichloromethane (100 ml) was treated with 1-hexylamine (2 ml, 2 . ح Spectrophotometry was used to monitor disappearance of the peak The solvent then removed under high vacuum, and the residue was crystallized from dichloromethane/hexane to give hexylamine 12% The reaction mixture (220 (25 ml) and The solvent was concentrated to 10 ml and left overnight in the refrigerator; reacted with dicyclohexylcarbodiimide (DCC) (400 mg, 1.75 mmol) The The dicyclohexylurea formed as a by-product was removed by The filtrate was concentrated and separated into these using mechanism of the formation of these compounds is shown in Figs. 2 preparation of purpurin-imide, the mixture of 6 and 7 (245 mg) was dissolved in ml), and a methanolic solution of KOH (0.5 mg/10 ml The reaction mixture was stirred for 5 min, 84 and mmol) 20 compounds were determined to be isoimide derivatives. to 6) stable. preparative plates (silica gel). The structure of for 0.34 derivatives 2 (major) and 3 (minor) as a mixture in nm and appearance of a new peak at 666 nm. under a nitrogen atmosphere with stirring for 12 h. mg, 0.34 mmol) was dissolved in dichloromethane isomers 4 and 5 (in the ratio of 1 temperature not the , <u>б</u>ш For 5 were (200 The yield was 90% (245 mg). yields, respectively (total: 220 mg). Purpurin-18 methyl ester 1 intermediate carbodiimides 4 and room at stirred was added. and filtration. individual and 4. THF (50 700 mmol) water) was at

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mmol)

and was monitored by spectrophotometry (appearance of a new peak The mixture was then diluted with dichloromethane The organic layer The from the solvent gave a residue which was chromatographed on a silica gel but were converted into the desired imides in one-pot without after 8 WAS For the syntheses of purpurin-imides 9-11 (see Table 2), the intermediate carbodiimides and isoimide analogs were not isolated, Evaporation of residue obtained dichloromethane/hexane, and the desired purpurin-imide column (elution with 2% acetone/dichloromethane). **crystallized** (100 ml) and washed with water (3 x 100 ml). was dried over anhydrous sodium sulfate. The from 1). Was appropriate eluates were combined. ក្ខ័ solvent obtained in 85% yield (185 the further purification. at 705 nm). evaporating amides, other

and Isoimide derivative of Bacteriopurpurin-a propyl ester (17) :(81)

obtained from R. spheroides was dissolved in dichloromethane (100 The the and the residue was crystallized from dichloromethane/hexane to 0.05 mmol), reaction mixture was stirred at room temperature for 24h; at Bl3nm (due to starting material) and appearance of a new The reaction mixture The solvent was then removed under high vacuum, as spectrophotometry was used to monitor the disappearance of mmol). (major) 0.1 , pm and 14 ml) and was treated with 1-hexylamine (0.2 ml, (30 Bacteriopurpurin-a propyl ester **12a** mixture in 10 and 90% yields respectively. give hexylamine derivatives 13 (minor) at 786nm. peak peak

The filtrate was concentrated and separated into ml) and solvent was concentrated to 10 ml and left overnight in the refrigerator; removed by 72% overall). On the basis of NMR data, these compounds were found to 0.25 individual isomers 17 and 18 (in the ratio of 1 to 9) The (25 (30 mg, 0.05 mmol) was dissolved in dichloromethane reacted with dicyclohexylcarbodiimide (DCC) (50 mg, Was under a nitrogen atmosphere with stirring for 12 h. preparative plates (silica gel). Yield: (28 mg, a by-product isoimide derivatives of bacteriopurpurin-a às formed dicyclohexylurea filtration.

using

å

Spectroscopic Data:

[Fisher's : (8) Ester Methyl Purpurin-18-N-hexylimide Nomenclature]

7a-647 (12,000); 549 (23,000); 510 (10,000); 483 (8,000); 417 (120,000). 8.58 (s, ô-meso H), 7.92 (dd, J 19.5, 12.8 Hz, 2a-H), 6.29 (dd, J 7-H), 4.48 (t, N-hexylimide-a-CH₂), 4.38 (g, J 8.0 Hz, 8-H), 3.84 5-Me), 3.62 (g, J 7.5 Hz, 4a-CH₂), 3.56 (s, OMe), 3.34 (s, 1--N (E) hexylimide-d,e-CH₂CH₂), 0.46 (t, J 7.8 Hz, N-hexylimide-f-CH₃), -(δ ppm, CDCl₃): 9.63 (s, β -meso H), 9.38 (s, α -meso H), (q 19.5 Hz, 2b-H), 6.18 (dd, J 12.8 Hz, 2b'-H), 5.37 (d, J 8.5 Hz, H), 2.06 (m, 7a'-H), 2.00 (m, N-hexylimide-b,c-CH₂CH₂), 1.74 (46,000); н Е J 7.2 Hz, 4-b Me), 1.43 Me), 3.18 (3-Me), 2.65 (m, 7b-H), 2.51 (m, 7b'-H), 2.40 0.08 and -0.17 (each br s, NH). m/z (LRMS): 661 (M+H) 705 Mp. 221-223°C. UV/Vis: (λ max/nm, ε): J 8.0 Hz, 8-Me), 1.65 (t, ¹H NMR (s)

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0 M

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: (6)

Ester

Dimethyl

Purpurin-18-N-hexylimide-7-N-aspartylamide

Purpurin-18-15¹-hexylisoimide methyl ester (7)

9.49 H), 7.92 (dd, J 19.4, 12.2 Hz, 2a-H), 6.31 (dd, J 19.4 Hz, 2b-H), 6.18 (dd, J 12.2 Hz, 2b'-H), 5.26 isoimide-CH₂), 3.83 (s, 5-Me), 3.76 (g, J 7.5 Hz, 4a-CH₂), 3.58 (s, OMe), 3.46 (s, 1-Me), 3.28 (3-Me), 2.65 (π, 7b-H), 2.58-2.00 (ш, 7b'-H; ш, 7а-Н; ш, 7a'-H; ш, hexyl isoimide-b,с-CH₂CH₂; 1.78 hexylamide-d,e-CH₂CH₂), 0.96 (t. J 7.8 Hz, hexyl isoimide f-CH₃), hexyl Ĕ $CDCl_3$): 9.73 (s, β -meso H), (d, J 8.0 Hz, 8-Me), 1.72 (t, J 7.2 Hz, 4-b Me), 1.61 J 8.0 Hz, 8-H), 4.10 (m, (H + W) -0.61 and -0.88 (each br s, NH). m/z (LRMS): 661 ppm, (d, J 8.5 Hz, 7-H), 4.57 (g, (s, α-meso H), 8.65 (s, ô-meso ¹H NMR (ó Mp. 138-139°C.

Purpurin-18- 13¹-hexyl isoimide methyl ester (6)

(s, OMe), 3.42 (s, 1-Me), 3.24 (3-Me), 2.65 (m, 7b-H), 2.51-2.00 5 2a-(d, J 8.5 Hz, 7-H), 4.52 (g, J 8.0 Hz, 8-H), 4.06 (t, hexyl isoimide-a-CH₂), 3.81 (s, 5-Me), 3.74 (q, *J* 7.5 Hz, 4a-CH₂), 3.56 ¹H NMR (δ ppm, CDCl₃): 9.74 (s, β -meso H), 9.57 H), 6.33 (dd, J 19.2 Hz, 2b-H), 6.15 (dd, J 12.5 Hz, 2b'-H), 5.24 hexylisoimide-b, c-CH₂CH₂; m, s, NH). ť ť (s, α-meso H), 8.75 (s, δ-meso H), 7.94 (dd, J 19.2, 12.5 Hz, 7.2 Hz, 4-b Me), 1.58 (m, hexyl isoimide-d,e-CH₂CH₂), 0.98 8-Me), 1.68 h 7.8 Hz, hexylisoimide-f-CH $_{\rm J}$), -0.66 and -0.84 (each H2, J 8.0 7a'-H; m, ď, dicyclohexylisourea-CH₂), 1.76 m, 7a-H; m, m/z (LRMS): 661 (M+H) Mp. 142-143°C. (m, 7b'-H;

[Fisher's Nomenclature] Mp. 218-219°C. UV/Vis: (λ max/nm, ε): 705 (43,200);

8.58 (s, ð-meso H), 7.90 (dd, J 19.8, 12.6 Hz, 2a-H), 6.98 (d, J 2b-H), 6.18 (dd, J z Ĕ, (43,200); 648 (11,000); 549 (21,000); 510 (9,200); 483 (7,800); 417 (112,000); NMR (δ ppm, CDCl₃): 9.60 (s, β -meso H), 9.34 (s, α -meso H), aspartate-CH), 3.82 (s, 5-Me), 3.69 (s, OMe), 3.64 (g, J 7.5 Hz, (m, 7a'-H), 1.99 (m, N-hexylimide-b, c-CH₂CH₂), 1.75 (d, J 8.0 Hz, 8+Me), 1.66 (t, J 7.5 Hz, 4-b Me), 1.45 (m, N-hexylimide-4a-CH₂), 3.61 (s, OMe), 3.36 (s, 1-Me), 3.16 (3-Me), 2.84 (m, aspartate-CH₂), 2.64 (m, 7b-H), 2.51 (m, 7b'-H), 2.46 (m, 7a-H), -0.38 and <u>ب</u> Hz, 8-H), 4.38 4.46 d,e-CH₂CH₂), 0.96 (t, J 7.8 Hz, N-hexylimide-f-CH₃), 7-Н), 9.6, aspartate-NHCO), 6.32 (dd, J 19.8 H2, 0.11 (each br s, NH). m/z (LRMS): 791 (M+H) Ηz, hexylisoimide-a-CH₂), 4.44 (q, *J* 8.0 8.5 Ь 2b'-H), 5.34 (đ, Н2, 2.06 12.6 1_H

Purpurin-18-N-hexylimide-7-N-Aspartylamide-di-tert-butyl Ester (10): Mp. 190-192°C. UV/Vis: (λ max/nm, ε): 705 (42,800); 648 (11,000); 549 (20,000); 510 (9,000); 483 (7,500); 417 (110,000). ¹H NMR (δ ppm, CDCl₃): 9.63 (s, β -meso H), 9.37 (s, α -meso H), 8.58 (s, δ -meso H), 7.86 (dd, J 19.4, 12.5 Hz, 2a-H), 6.74 (d, J 9.6 Hz, aspartate-NHCO), 6.32 (dd, J 19.4 Hz, 2b-H), 6.21 (dd, J 19.6 Hz, aspartate-NHCO), 6.32 (dd, J 19.4 Hz, 2b-H), 7.84 (s, H), 4.41 (t,N-hexylimide-CH₂), 3.99 (m, aspartate-CH), 3.84 (s, 5-Me), 3.68 (q, J 7.5 Hz, 4a-CH₂), 3.36 (s, 1-Me), 3.18 (3-Me),

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2.76 (m, aspartate-CH ₂), 2.65 (m, 7b-H), 2.51 (m 7a-H) 2.06 (m 7a'H) 1.92 (m N-bevv)	7b-H), 2.51 (m, 7b'-H), 2.46 (m N-hevvlimide-b.c-CH.CH.1	H), 4.30 (m, 2H, 3-H, 18-H), 4.08 (m, 1H, 8-H), 3.94	(t, 2H,
8 (d, J 8.0 Hz, 8-Me), 1.66 (t,	7.5 Hz, 4-b Me), 1.37 (s,	1 ₂), 3.66 (s, 3H, 12-Me), 3.55 (s, 3H, 2-Me), 3	3H, 3-
aspartate- ^t Bu), 1.34 (s, aspartate- ^t Bu), 1.14	Bu), 1.14 (m, N-hexylimide-	ме), 2./3 (m, H, 17b-H), 2.41 (m, 5H, CH ₂ CH ₂ CH ₃ + 8a-CH ₂ + 7b′- H), 2.14 (m, H, 17a-H), 1.98 (m, H, 17a'-H), 1.81, 1.73 (each d	+ 7b'- ach d.
d,e-CH ₂ CH ₂), 0.95 (t, J 7.8 Hz, N-hexylimide-f-CH ₃), -0.38 0.11 (each br s, NH). Mass: m/z (HRMS): require:	kylimide-f-CH ₃), ~0.38 and - m/z (HRMS): requires for	3H, J = 8.0, 18-Me, 7-Me), 1.11 (t, 3H, J =́ 7.8, 3-b Me),	0.83
7N607: 875.5071. Found 875.5016		(t, 3H, $J = 8.2$, CH_2CH_3), -0.30 and -0.67 (each br s, 23-NH).	2H, 21,
Purpurin-18-N-glycylimide-tert-Butyl Ester-7 tert-butyl Ester (11): [Fisher's Nomenclature]	Ester-7-aspartylamide-di- nclature)	Bacteriopurpurin-a+15 ¹ -N-hexylisoimide (17): {F.	[Fisher's
Mp. 138-139°C. UV/Vis: (λ max/nm,	, ɛ): 705 (41,300); 649	ure):	
,000); 549 (19,000);	417 (10	00//12: AMAX: /95 (5/,000); 53/ (24,500); 410 (50,400); (89,600). NMR (CDCl ₃ , ô PPM): 9.21 (s, 1H, 5-H), 8.77 (s.	0); 363 (s. 1H.
[±] H NMR (δ ppm, CDCl ₃): 9.61 (s, β-meso H), 9.35 (s, 8.56 (s. δ-meso H) 7.88 (dd J 19.5. 12.6 Hz. 2a-H).	so H), 9.35 (s, α-meso H), 12.6 Hz. 2a-H), 6.65 (d. J	2H, NHCO + 17-H),	
9.5 Hz, aspartate-NHCO), 6.26 (dd, J 1	5.16 (dd,	3-H, 18-H), 4.11 (m, 3H, 8-H + hexylamide-a-CH ₂),	3.91 (t,
12.5 Hz, 2b'-H), 5.27 (d, J 8.5 Hz, 7-H), 5.18	-H), 5.18 (q, glycine-CH ₂),	(s, 3H, 2-Me),	3.19 (s,
4.68 (q, J 7.5 Hz, 8-H), 4.38 (m, asp	3.38 (s,	э-ме), гюс (m, н, 1/ю-н), 2.44 (m, 5H, CH ₂ CH ₂ + -H), 2.14 (m, 6H, 17а-H + hexvlisoimide-b.c-CH ₂ +	8a-CH ₂ + 17a'-H)
3.64 (q, J 7.5 Hz, 4a-CH ₂), 3.35 (s, 1-Me), 3.16	-Me), 3.16 (3-Me), 2.79 (m,	.84 (each d, 3H, J = 8.0, 18-Me, 7-Me), 1.60	(m, 4H,
aspatuace-cn2/; 2:00 (m, /b-n); 2:04 (m, /b -n); 2: 2:01 (m, 7a'-H), 1:73 (d, J 7.5 Hz, 8-Me), 1.67 (t,	с. J 7.5 Н	hexylamide- d ,e-CH ₂ , 1.11 (t, 3H, $J = 7.8$, 3-b Me), 0.97 (t,	, нс ,
Me), 1.58 (s, glycine- ^t Bu), 1.38 (s, aspartate- ^t	Bu), 1	amide-f-CH ₃)	and -
aspartate- ^t Bu), 0.10 and -0.04 (each br	br s, NH). m/z (LRMS) 905.4	1.03 (each br s, 2H, 21, 23-NH). Mass: LRMS:708(M+H)	
(H+W) .		Bacteriopurpurin-a-13 ¹ -N-hexyl isoimide (18) (Fi	[Fisher's
Bacteriopurpurin-a 17-propyl Ester (12a): [Fisher's Nomenclature]	a): [Fisher's Nomenclature]		
.2, Àmax, л	,000); 543 (32,000); 408 &	cl ₃ , ô ppm): d'H: 9.38 (s, 1H, 5	;(004,8c) -H), 8.88
8.79 (s, 1H, 10-H), 8.62 (s, 1 H, 20-H),	, 5.14 (d, 1H, J=8.	(s, 1H, 10-H), 8.73 (s, 1H, 20-H), 5.46 (m, 1H, NHCO), 5.18	8 (d,
21		22	

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1H, J = 8.0, 17-H), 4.34 (m, 2H, 3-H, 18-H), 4.17 (m, 1H, 8-H), 3.91 (m, 2H, hexylisoimide- $a-CH_2$), 4.06 (t, 2H, CO_2CH_2), 3.68 (s, 3H, 12-Me), 3.59 (s, 3H, 2-Me), 3.19 (s, 3H, 3-Me), 2.73 (m, H, 17b-H), 2.42 (m, 5H, CH_2CH_3 + $8a-CH_2$ + 7b'-H), 2.14 (m, H, 17a-H), 2.08 (m, 5H, hexylamide-b, $c-CH_2+17a'-H$), 2.01, 1.93 (each d, 3H, J=8.0, 18-Me, 7-Me), 1.57 (m, 4H, hexylisoimide-d, $e-CH_2$), 1.12 (t, 3H, J = 7.8, 3-b Me), 0.96 (t, 3H, hexylamide- $f-CH_3$), 0.87 (t, 3H, J = 8.2, $CH_2CH_2CH_3$), -0.86 and -1.13 (each br s, 2H, 21, 23-NH). Mass: LRMS:708 (M+1)

Biological Studies:

Determination of drug uptake:

The absorption spectrum of a compound in living tissue can be recorded using an instrument and technique which we have through the tissue. The light originates in a high pressure Xenon arc lamp and passes through a grating monochromator to a 90 The distal end of this fiber is placed in contact with the tissue The noninvasive character of this measurement makes data collection possible at a number of time points after the i.v. injection of an experimental The light signal is detected by a photodiode. The photo current The experiment measures the light which scatters (e.g., an experimental mouse tumor) and the light is collected by second fiber placed in contact with the tissue at a fixed H2) light absorbing compound (e.g., a potential photosensitizer). 06) Hz chopper and then into a 400 $\mu {
m m}$ diameter guartz fiber. is converted into a voltage, amplified by a tuned distance (3 to 5 mm) from the first fiber. developed. a

amplifier and synchronously detected. The chopping at 90 Hz and synchronous detection makes examining spectra under normal room lighting possible.

The drug was then administered by tail vein or these experiments, the mice were first anesthetized The optical vitro The second spectrum contains a component caused by the presence of the drug in the This in vivo drug absorption spectrum is best displayed double beam absorption spectrophotometer. The pre-injection The ratio of these two spectra is normalized by dividing the signal strength at a wavelength where power as a function of wavelength was recorded before the i.v. to the by taking the ratio of the post-injection spectrum to the preinjection spectrum. This ratio offers the same advantages as a mouse data can be thought of as the reference beam sample data as reference beam plus certainly not influenced by the wavelength dependence of the light signal which characterizes the instrument. As a safeguard against day to day or hour to hour drift in the total light output of the lamp, both spectra (pre- and post-injection) are expected longest wavelength of the experimental drug's in injection of the sensitizer. The monochromator is set (typically a cuvette and solvents) and the post-injection using either Pentobarbital or Ketamine Xylazine i.p. the sample beam containing everything in the injection and the light signal recorded. the drug absorption is negligible. experimental drug. absorption spectrum. tumor. the

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Photosensitizing Efficacy: Part I - In vivo Efficacy of Bacteriochlorins 17 and 18 using SMT-F Tumor Model

The new photosensitizer was screened in a mouse/tumor model system. A model system consisted of observing the size reduction of the SMT-F tumor, a fast growing spontaneous mouse mammary tumor subline, transplanted subcutaneously to male or female DBA/2 HA-DD mice. The tumor line was maintained in vivo via serial transplantation in the same mouse strain. DBA/2 Ha-DD mice are readily available and were obtained locally.

age (approximately 6 20 g), small pieces of tumor (1mm cube) were transplanted with a 18 gauge trocar from a donor This technique provides for relatively uniform tumor size and allows location of the tumor in the right When tumor reached 4-5 mm in diameter, the animals were injected described above. Prior to irradiation, the fur overgrown and Three or twenty four hours after injecting the drug, the mouse was group auxiliary region of the animal within each experimental group. Only animals with single tumors were chosen for experiments. chosen from the surrounding the tumor was removed with electric clippers. When mice were both the appropriate with the potential photosensitizer placed in a custom-made aluminum holder weeks) and weight (approximately tumor to recipient mouse.

Irradiation Conditions:

Standard light dose was 75 mW/cm² for 30 min for a total incident dose of 135 J/cm^2 from a tunable dye laser tuned to the maximum red absorption peak. Spectra Physics 2040, a guartz

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fiber fitted with a microlens was interfaced to the dye laser deliver a uniform field of light. Laser output was measured with a power meter. Further studies at various light doses and treatment conditions are currently in progress.

Experimental Procedure:

Following light exposure, the mice were kept in groups of 5 per cage and supplied with pelleted food and tap water ad libitum. Tumor size and gross appearance of both tumor and overlying surrounding skin was monitored daily for 80 days after photoillumination unless growth of non-responsive tumor require early sacrifice of those animals.

The the the The photosensitizer was dissolved in known guantity of Tween 80 (Aldrich) surfactant and diluted by a factor of 10 with saline 10-20%. Absorption spectra were obtained at on the basis of filter. of the photosensitizer solution to produce a final Tween 80 concentration of a syringe using a Spectronic Genesis5 spectrophotometer. concentration of the solution was determined The solution was then filtered through extinction coefficient value longest wavelength absorption.

Before injecting the drug into mice, the purity of the compounds was ascertained by analytical HPLC using Spectra Physics HPLC, connected with C8 reverse phase column, eluted with methanol/water by adjusting the pH to 7.0 using phosphate buffer.

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The time for growth

Tumor

all,

out with tumor-bearing mice which received no treatment at

or received light or photosensitizer only.

to the tumor to 400 mm³ was then estimated by interpolation of

tumor and w is the axis perpendicular to l.

the times just before and after 400 mm 3 was reached.

using the formula $v=\{lw^2\}/2$, where l is the longest axis of the

response was recorded on the basis of the number of animals which were found to be tumor free. Appropriate controls were carried

Alkylimide derivatives of bacteriochlorins:

Procedures similar to preparation of N-hexylimide derivatives were followed except that various other alkyl amines were substituted for hexylamine.

The resulting compounds were tested for in vivo antitumor activity.

4-6 mm diameter tumors (DBA/2 mice transplanted with SMT-F tumors) were exposed to 75 mW/cm² for 30 minutes to deliver light (135 J/cm²) from a tunable dye laser tuned to a maximum red absorption peak. The tumors were non-palpable and five mice were used per group.

The results are in Table 4. The results indicate a large suppressive effect for isomers where the extended alkyl group is closest to the amino acid group.

Part II - In vivo efficacy of bacteriochlorins 17 and 18 using Radiation Induced Fibrosarcoma (RIF) Tumor Model.

In brief, six mice per group the As shown in Table 3, the animals were treated at and at least every other day thereafter, tumors were measured in orthogonal diameters with an electronic caliper (ultra-Cal Mark automatically recorded, where the tumor volume, V, was calculated A mixture of bacteriochlorins 17 and 18 was also evaluated Each measurement was for in vivo PDT efficacy using another model (RIF tumor moded) were chosen for Beginning 24h after PDT, (uu III; Fred V. Fowler Co., Boston MA). with appropriate tumor size (4-5 routinely used in our laboratory. variable doses of light and drug. experiment.

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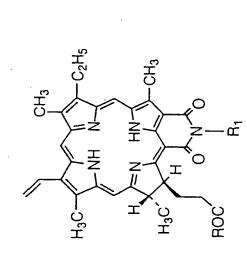
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Method No. Conditions Yield (%) imide 4 anhydride 1 starting materials (5 &6) 1. THF, refluxing for 4h. 5 70 25 2. Imidazole, 140°C, 1h Decomposition products 25 3. K-10 Clay, CH2Cl2, 24h 10-12 80-85 0 4. CH2Cl2, 10 days 15-20 20-25 50 5. DCC with: 0 0 0 100 a. K-10 Clay, RT 0 0 100 a. K-10 Clay, RT 0 0 100 a. K-10 Clay, RT 0 0 0 100 b. DCC with: 0 0 0 100 a. K-10 Clay, RT 0 0 0 0 b. DCC with: 0 0 0 0 0 a. K-10 Clay, RT 0 0 0 0 0 0 b. DCC with: 0 0 0 0 0 0 0 0 <t< th=""><th></th><th>at var</th><th>at various reaction conditions</th><th>conditions</th><th></th></t<>		at var	at various reaction conditions	conditions	
IHF, refluxing for 4h. 5 Idazole, 140°C, 1h Decompos -10 Clay, CH2Cl2, 24h 10-12 -10 Clay, RT 0 DBU, RT 0	Acthod No.	Conditions		Yield (9	R.)
THF, refluxing for 4h. 5 70 nidazole. 140°C. 1h Decomposition products -10 Clay. CH2Cl2. 24h 10-12 80-85 -10 Clay. CH2Cl2. 24h 10-12 80-85 CH2Cl2. 10 days 15-20 20-25 CH2Cl2. 10 days 15-20 20-25 BU, RT 0 0 0 DBU, RT 0 0 0 RT, 10 min 85 0 0 DBU, RT 0 0 0 DBU, RTF 60°C 30 0 0			imide 4	anhydride 1	starting materials (5 &
nidazole, 140°C, 1h Decomposition products -10 Clay, CH ₂ Cl ₂ , 24h 10-12 80-85 CH ₂ Cl ₂ , 10 days 15-20 20-25 K-10 Clay, RT 0 0 0 DBU, RT 0 0 0 DBU, toluene, reflux 2h 60 0 KOHMACOH 85 0 DBU, toluene, reflux 2h 10 0	. THF	refluxing for 4h.	5	70	25
-10 Clay. CH ₂ Cl ₂ , 24h 10-12 80-85 CH ₂ Cl ₂ , 10 days 15-20 20-25 K-10 Clay. RT 0 0 0 DBU, RT 0 0 0 DBU, RT 0 0 0 CHPMcOH 85 0 0 KCHPMcOH 85 0 0 DBU, RT 0 0 0 DBU, RT 0 0 0 DBU, RT 600C 30 0	lmida .	azole, 140ºC, 1h	Decomp	osition products	
CH ₂ Cl ₂ , 10 days 15-20 20-25 K-10 Clay, RT 0 0 DBU, RT 0 0 DBU, IRT 0 0 DBU, IRT 0 0 RT, 10 min 85 0 DBU, RT 0 0 DBU, RT, 10 min 85 0 DBU, RT, 10 min 30 0	K-10	Clay, CH ₂ Cl ₂ , 24h	10-12	80-85	0
K-10 Clay, RT 0 0 DBU, Ioluene, reflux 2h 85 0 KCH/MeOH 85 0 DBU, RT, 10 min 85 0 DBU, RT 0 0 DBU, colume, reflux 2h 10 50 DBU, tolume, reflux 2h 10 50 DBU, tolume, reflux 2h 10 50 DBU, THF 60°C 30 0	. CH	1 ₂ Cl ₂ , 10 days	15-20	20-25	50
K-10 Clay, RT 0 0 DBU, RT 0 0 DBU, RT 0 0 DBU, toluene, reflux 2h 60 0 RC0H/MeOH 85 0 RT, 10 min 85 0 DBU, RT 0 0 DBU, tolune, reflux 2h 10 50 DBU, tolune, reflux 2h 10 50 DBU, tolune, reflux 2h 10 50 DBU, THF 60°C 30 0	. DCC with:	• • • • • • • • • • • • • • • • • • • •			
DBU, RT 0 0 DBU, toluene, reflux 2h 60 0 KOHMeOH RT, 10 min 85 0 DBU, RT 0 0 DBU, tolune, reflux 2h 10 50 DBU, THF 60°C 30 0		10 Clay, RT	0	0	100
DBU, toluene, reflux 2h 60 0 KOH/MeOH RT, 10 min 85 0 DBU, RT 0 0 DBU, tolune, reflux 2h 10 50 DBU, THF 60°C 30 0		IU, RT	0	0	100
RUFMACUA RT. 10 min 85 0 DBU, RT 0 0 DBU, tolune, reflux 2h 10 50 DBU, THF 60°C 30 0	C. DB	U, toluene, reflux 2h	60	0	40
DBU, RT 0 0 DBU, tolune, reflux 2h 10 50 DBU, THF 60ºC 30 0	d. KU RT,	H/MeUH	85	0	0
DBU, RT 0 0 DBU, tolune, refux 2h 10 50 DBU, THF 60°C 30 0 KOHMACH 30 0	TCD with:				
DBU tolune, reflux 2h 10 50 DBU THF 60ºC 30 0 KOHAM-OH		IU, RT	0	0	001
00C 30 0	b. DB(U, tolune, reflux 2h	10	50	
	c. DB(U, THF 60°C	30	0	60
10 0	d. KUI RT,	HMEON 10 min	10	0	06

Table 2. Yields of purpurin-imides



Compound No.	R	R1 % yield	% yield
80	1	n-hexy!	85
6	Asp-di-methyl ester	n-hexyl 89	89
10	10 Asp-di- <i>teri</i> - butyl ester n-hexyl 87	n-hexyl	87
11	Asp-di-terr- butyl ester terr-butyl Gly 78	rerr-butyl Gly	78

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onse	14	808	
Tumor Response days	7	100\$	
άn L	1-2	100\$	
Timer after Injection hrs		24	, c
Light Dose Rate mW/cm ²		75	10
Drug Dose mmol/kg		0.20	0.20

	14	80\$	40\$	808	60\$	
days	7	100\$	60\$	100\$	80\$	
	1-2	100\$	808	100\$	80\$	
SJU UOTODA(IIT		24	24	24	24	
		75	30	75	30	
		0.20	0.20	0.25	0.25	

Comparative *in vivo* Antitumor Activity of Bacteriochlorins Tumor response (d)^{1,*} Time(h) in vivo Dose

Compound

17. R = (CH₃,5CH₃ 19. R = (CH₃),6CH₃ 21. R = (CH₂),2CH₃ 21. R = (CH₂),2CH₃ 23. R = (CH₂),9CH₃

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(MAJOR (B04 NM)

MINOR (795 NM)

CO₂C₃H,

	30+	40 41 42 43 44		
	15	REGROWTILON DAY 15	NO RESPONSE	REGROWTH ON DAY 4
	1-2			REGR
(µmol/kg) absorption betw. injection	and light treatment	24	0.47 804 24	24
absorption	(У тах)	804	804	804
(Jumol/kg)		xture of 0.47 and 18	0.47	0.47
		Mixture of 0.47 17 and 18	20	22

\$ 8 5 804 0.47 Mixture of 23 and 24

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¹ 4-5 mm diameter tumors were exposed to 75 MW/cm² for 30 min to deliver 135 J/cm² light from a tunable dye laser tuned to the maximum red absorption peak. d= days. [★] Non-palpable tumors.

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WHAT IS CLAIMED IS:	where R is selected from the group consisting of OMe and Asp-di-
 A method for the manufacture of an imide derivative of 	ester and R ₁
purpurin comprising:	hexyl and tert-butyl Gly.
reacting hexylamine with a chlorin or bacteriochlorin having	4. The method of Claim 1, wherein the imide derivative is
a macrocycle with a six membered anhydride ring fused thereto,	further reacted with an alkali metal hydroxide to obtain a cyclic
said macrocycle containing a and b rings which may be saturated	imide.
or unsaturated at ${ extsf{R}_4}$ to ${ extsf{R}_{11}}$ positions of the rings and which ${ extsf{R}_4}$	
and R ₁₁ positions may contain at least one group selected from the group consisting of hydrogen budrowy formal substituted	thod of Claim 1.
unsubstituted alkyl, alkoxy, alkenyl, aryl an	6. An imide of purpurin manufactured in accordance with the
wherein carbon containing groups may be substituted with a	
substituent selected from carboxyl, hydroxy, phosphoro, carboxy,	
halo, sulfo, amino and ether, to obtain a purpurin derivative;	7. A reaction product comprising an imide of purpurin
and reacting the purpurin derivative with a carbodiimide to	manufactured in accordance with the method of Claim 3.
obtain the imide derivative of purpurin.	⁸ . À reaction product comprising an imide of purpurin
2. The method of Claim 1, wherein the carbodiimide is	manufactured in accordance with the method of Claim 4.
dicycloberylcarbodiimide.	9. A compound of the formula:
3. The method of Claim 1, wherein the imide derivative is	
further reacted with an alkali metal hydroxide to obtain a	
purpurin imide of the formula:	
CH3	R1, A NH N R4
H ₃ C C H ₅ C C H ₅	HM HM
HZ I	H.C.
H, CH HN HN HN	H,
	RIC Z O Z
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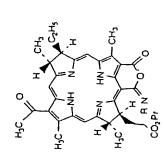
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R₁₄ is where z is = 0 or = NR_{14} ; R_{1} is an amino acid group, a polyamine group; R_4 through R_{11} are -H, -OH, alkyl, alkylene, -O R_{16} , where an amino acid R_{16} is H, alkyl or aryl, or a carbonyl containing group, provided may be taken together with $m R_7$ to form =0; $m R_8$ may be taken together with $^{
m R_9}$ to form =0; $^{
m R_{10}}$ may be taken together with $^{
m R_{11}}$ to form =0; and together form a chemical bond; and ${ extsf{R}}_{12}$ is hydrogen or lower ${ t R}_{ extsf{d}}$ and ${ t R}_{ extsf{7}}$ may together form a chemical bond and ${ t R}_{ extsf{8}}$ and ${ t R}_{ extsf{11}}$ may alkyl; provided that if one z is = 0, the other z is = NR_{14} . to form =0; R₆ group, a polyether group or OR_{13} where R_{13} is alkyl; ч С polyamine group, R₄ may be taken together with R₅ alkyl, substituted alkyl, a that;

The compound of Claim 9 wherein R_{11} and R_{12} are -CH $_3$. The compound of Claim 9 wherein R_2 is an allyl group. 10. 11.

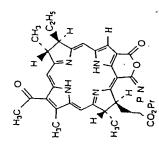
The compound of Claim 9 wherein R_3 is an allyl group. 12.

The compound of Claim 9 having the formula: 13.

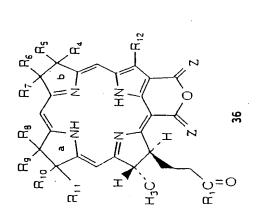


where R is normal alkyl of 2 through 12 carbon atoms.

The compound of Claim 9 having the formula: 14.



19. A method for the manufacture of an isoimide derivative of purpurin comprising reacting a purpurin of the formula: where R is normal alkyl of 2 through 12 carbon atoms. The compound of Claim 10 wherein R_2 is $0=COR_{15}$. The compound of Claim 15 wherein R_1 is H_3CO_2C- . The compound of Claim 16 wherein R_2 is $H_3CO_2C^-$. The compound of Claim 10 wherein R_3 is 0=COR₁₅. 15. 16. 17. 18.



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wherein ${f R}_1$ is an amino acid group, a polyamine group, a polyether group or OR_{13} where R_{13} is alkyl; R_{14} is alkyl, or substituted þ to form =0; R₆ may be taken together with тау ъ

to form =0; R_{10} alkyl, a polyamine group, or an amino acid group; ${
m R_4}$ through ${
m R_{11}}$ -OH, alkyl, alkylene, -OR $_{16}$, where R_{16} is H, alkyl or together form a chemical bond and ${
m R}_{
m g}$ and ${
m R}_{
m 11}$ may together form a chemical bond; and \mathbb{R}_{12} is hydrogen or lower alkyl; provided that if one z is =0, the other z is =NR $_{14}$, with 1-hexylamine to open the anhydride ring followed by reaction with a carbodiimide to R₇ may to form =0; and R_4 and aryl, or a carbonyl containing group, provided that; R₈ may be taken together with R₉ obtain the isoimide derivative of purpurin. be taken together with R₁₁ taken together with R₅ form =0; are -H, ç тау R7

A method for the manufacture of purpurin imide comprising reacting the compound of Claim 9 with alkali metal hydroxide. 20.

comprising

imide

manufacture of purpurin

the

for

A method

21.

A method for the manufacture of purpurin imide comprising A method for the manufacture of purpurin imide comprising reacting the compound of Claim 10 with an alkali metal hydroxide. reacting the compound of Claim 15 with an alkali metal hydroxide. 23. 22.

comprising comprising reacting the compound of Claim 17 with an alkali metal hydroxide. A method for the manufacture of purpurin imide 24. A method for the manufacture of purpurin imide 25.

reacting the compound of Claim 16 with an alkali metal hydroxide.

reacting the compound of Claim 18 with an alkali metal hydroxide.

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26. A method for the manufacture of purpurin imide comprising reacting the purpurin derivative from Claim 19 with alkali metal hydroxide

27. A method for the manufacture of purpurin imide comprising reacting the purpurin derivative from Claim 19 with alkali metal hydroxide

imide derivative of ал method for the manufacture of purpurin comprising: A 28.

R11 positions may contain at least one group selected from the group reacting hexylamine with a chlorin or bacteriochlorin having said macrocycle containing a and b rings which may be saturated substituted and aryl and aryloxy, wherein a substituent selected from carbonyl, hydroxy, phosphoro, carboxy, halo, sulfo, a macrocycle with a six membered anhydrid ring fused thereto, t t Р. to R₁₁ positions and which amino and ether, to obtain a purpurin derivative; and be substituted with consisting of hydrogen, hydroxy, formal, unsubstituted alkyl, alkoxy, alkenyl, carbon containing groups may unsaturated at R₄ 占

đ with derivative carbodiimide to obtain the imide derivative of purpurin. purpurin reacting the obtained

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AMENDED CLAIMS [received by the International Bureau on 9 September 1997 (09.09.97); original claims 1, 2, 9, 11, 17 and 19 amended; original claims 12, 15, 16, 18, 22, 23 and 25 cancelled; remaining claims unchanged (6 pages)]	R is selected 1 ester and R ₁
 A method for the manufacture of an imide derivative of purpurin commrisiant. 	Xyl and tert-butyl Gly.
reacting hexylamine with a chlorin or bacteriochlorin having	
macrocycle with a six membered anhydride ring fused thereto,	imide.
said macrocycle containing a and b rings which may be saturated or unsaturated at \mathbb{R}_4 to \mathbb{R}_{11} positions of the rings and which \mathbb{R}_4 and \mathbb{R}_{11} positions at least one group selected from	5. An imide of purpurin manufactured in accordance with the method of Claim 1.
group consisting of hydrogen, hydroxy, formyl, substituted unsubstituted alkyl, alkoxy, alkenyl, aryl and aryloxy	6. An imide of purpurin manufactured in accordance with the method of Claim 2.
wherein carbon containing groups may be substituted with a substituent selected from carboxyl, hydroxy, phosphoro, carboxy, halo, sulfo, amino and ether, to obtain a purpurin derivative;	7. A reaction product comprising an imide of purpurin manufactured in accordance with the method of Claim 3.
and reacting the purpurin derivative with a carbodiimide to obtain the imide derivative of purpurin.	B. A reaction product comprising an imide of purpurin manufactured in accordance with the method of Claim 4.
2. The method of Claim 1, wherein the carbodiimide is dicyclohexylcarbodiimide.	9. A compound of the formula:
3. The method of Claim 1, wherein the imide derivative is further reacted with an alkali metal hydroxide to obtain a purpurin imide of the formula:	R ₁₀ A A A A A A A A A A A A A A A A A A A
	H ₁ C, H ₁₂
HJC HJCHJCHJCHJCHJCHJCHJCHJCHJCHJCHJCHJCHJCH	
39 R; AMENDED SHEET (ARTICLE 19)	40 Amended Sheet (Article 19)

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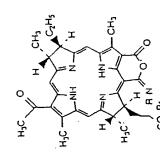
14.

where z is = 0 or = NR_{14} ; R_1 is an amino acid group, a polyamine a polyether group or OR_{13} where R_{13} is alkyl; R_{14} is alkyl, substituted alkyl, a polyamine group, or an amino acid substituted with sulfo, amino and ether substituents, provided that; ${
m R}_4$ may be taken together with group; R₄ through R₁₁ are -H, -OH, alkyl, alkoxy, alkenyl, R_{S} to form =0; R_{S} may be taken together with R_{7} to form =0; R_{8} alkylene, aryl, or aryloxy, or a carbonyl containing group, may be taken together with R_9 to form =0; R_{10} may be taken together with $extsf{R}_{11}$ to form =0; and $extsf{R}_{4}$ and $extsf{R}_{7}$ may together form a chemical bond and \mathbb{R}_8 and \mathbb{R}_{11} may together form a chemical bond; and R_{12} is hydrogen or lower alkyl; provided that if one z is halo, wherein carbon containing groups may be carbonyl, hydroxy, phosphoro, carboxy, 0, the other z is = NR_{14} . group,

10. The compound of Claim 9 wherein R_{11} and R_{12} are -CH₃.

11. The compound of Claim 9 wherein R_9 is -CoCH₃.

13. The compound of Claim 9 having the formula:

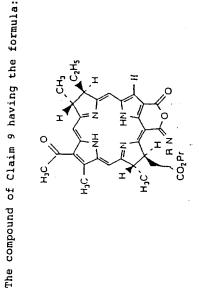


where R is normal alkyl of 2 through 12 carbon atoms.

AMENDED SHEET (ARTICLE 19)

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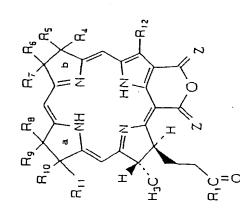
AMENDED SHEET (ARTICLE 19)



where R is normal alkyl of 2 through 12 carbon atoms.

17. The compound of Claim 9 wherein R_1 is $H_3CO_2C^2$.

19. A method for the manufacture of an isoimide derivati purpurin comprising reacting a purpurin of the formula:



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wherein z is =0 or NR_{14} ; R_1 is an amino acid group, a polyamine a polyether group or OR_{13} where R_{13} is alkyl; R_{14} is or substituted alkyl, a polyamine group, or an amino acid and group; R₄ through R₁₁ are -H, -OH, alkyl, alkoxy, alkenyl, alkylene, aryl, or aryloxy, or a carbonyl containing group, with ether substituents provided that; ${ t R}_4$ may be taken together with $^{
m R_5}$ to form =0; $m R_6$ may, be taken together with $m R_7$ to form =0; $m R_8$ taken together with R_9 to form =0; R_{10} may be taken together with ${
m R}_{11}$ to form =0; and ${
m R}_4$ and ${
m R}_7$ may together form a the other z is $=NR_{14}$, with 1-hexylamine to open the anhydride ring followed by reaction with a carbodiimide to obtain the chemical bond and $extsf{R}_{ extsf{l}}$ and $extsf{R}_{ extsf{l}1}$ may together form a chemical bond; and R_{12} is hydrogen or lower alkyl; provided that if one z is =0, sulfo, amino substituted carbonyl, hydroxy, phosphoro, carboxy, halo, wherein carbon containing groups may be isoimide derivative of purpurin. may be group, alkyl,

20. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 9 with alkali metal hydroxide.

21. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 10 with an alkali metal hydroxide.
24. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 17 with an alkali metal hydroxide.
26. A method for the manufacture of purpurin imide comprising

reacting the purpurin derivative from Claim 19 with alkali metal

hydroxide

27. A method for the manufacture of purpurin imide comprising reacting the purpurin derivative from Claim 19 with alkali metal hydroxide.

43 Amended Sheet (Article 19)

AMENDED SHEET (ARTICLE 19)

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28. A method for the manufacture of an imide derivative of purpurin comprising: reacting hexylamine with a chlorin or bacteriochlorin having a macrocycle with a six membered anhydrid ring fused thereto, said macrocycle containing a and b rings which may be saturated or unsaturated at R_4 to R_{11} positions and which R_4 to R_{11} positions may contain at least one group selected from the group consisting of hydrogen, hydroxy, formal, substituted and unsubstituted alkyl, alkoxy, alkenyl, aryl and aryloxy, wherein carbon containing groups may be substituted with a substituent selected from carbonyl, hydroxy, phosphoro, carboxy, halo, sulfo, amino and ether, to obtain a purpurin derivative; and

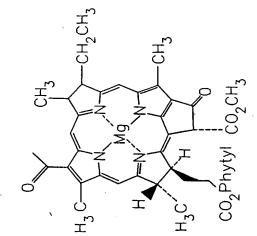
reacting the obtained purpurin derivative with a carbodiimide to obtain the imide derivative of purpurin.

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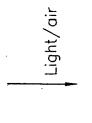
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N=0-0

Bacteriochlorophyll-a, 780 nm



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В=CONH(CH^S)²C

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3. B = COOH, $B^{1} = R^{1}$, $B^{1} = R^{1}$, $B^{1} = COOH$ (Major) $H^{2}CO_{2}C$, $B^{1} = COOH$ (Major) $H^{2}CO_{2}C$, $B^{1} = COOH$ (Major)

ΗN

N=2-0-2

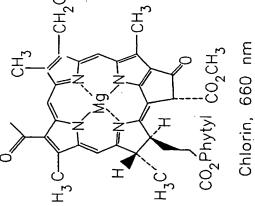
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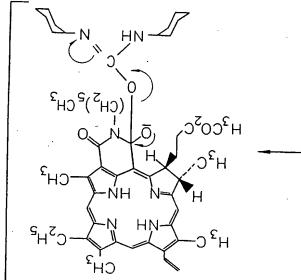


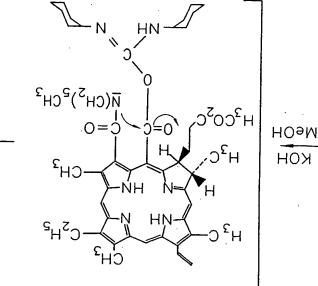
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FORMATION OF CYCLIC IMIDE







FORMATION OF CYCLIC ANHYDRIDE



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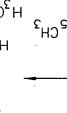
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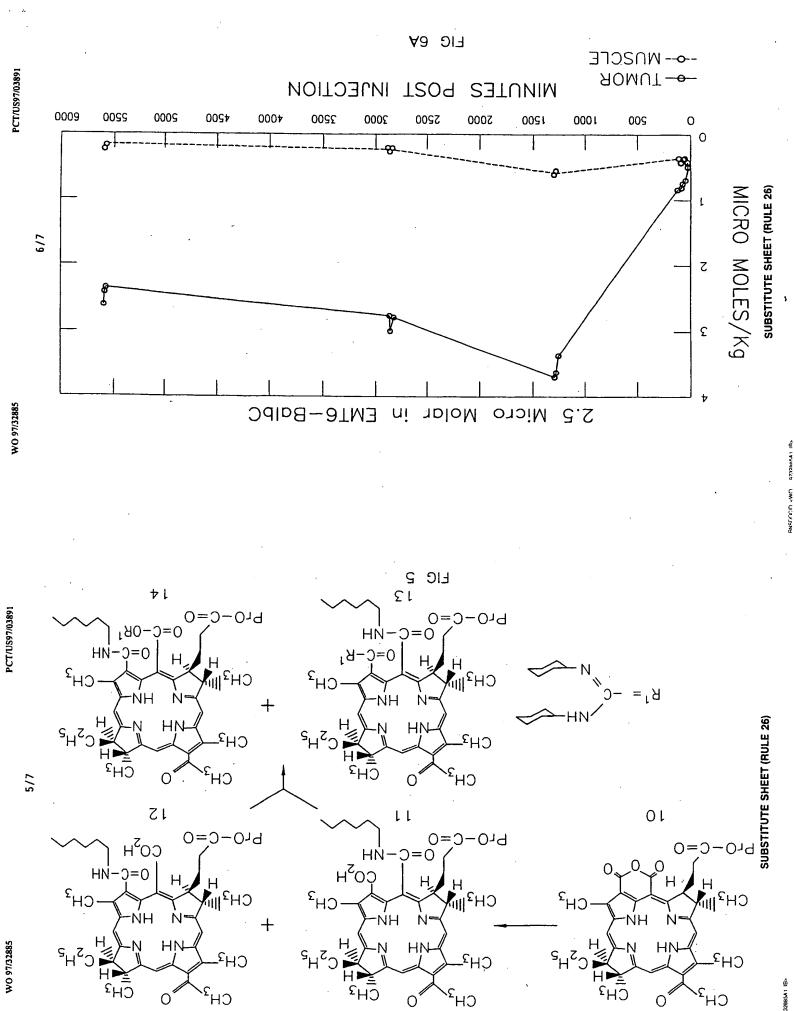
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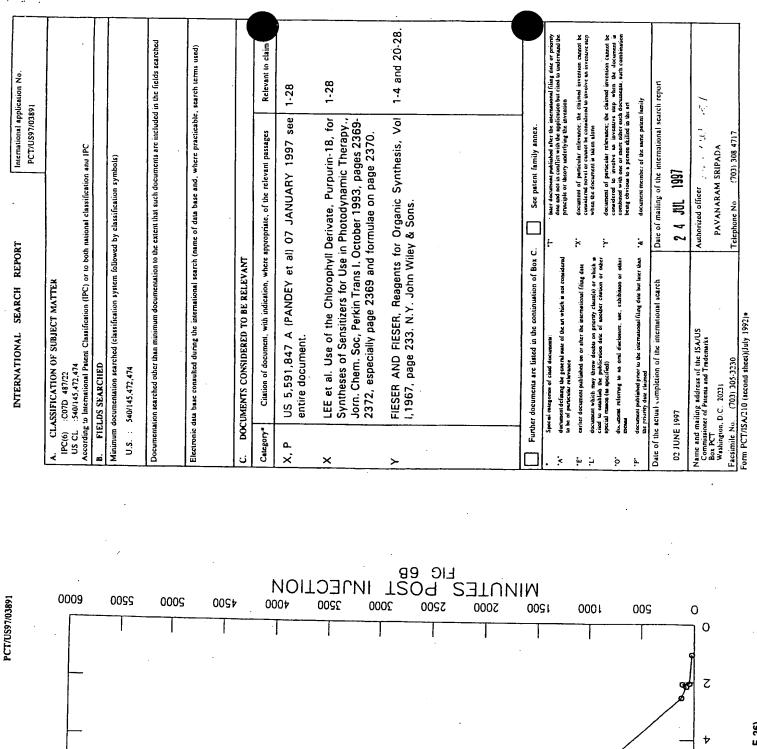
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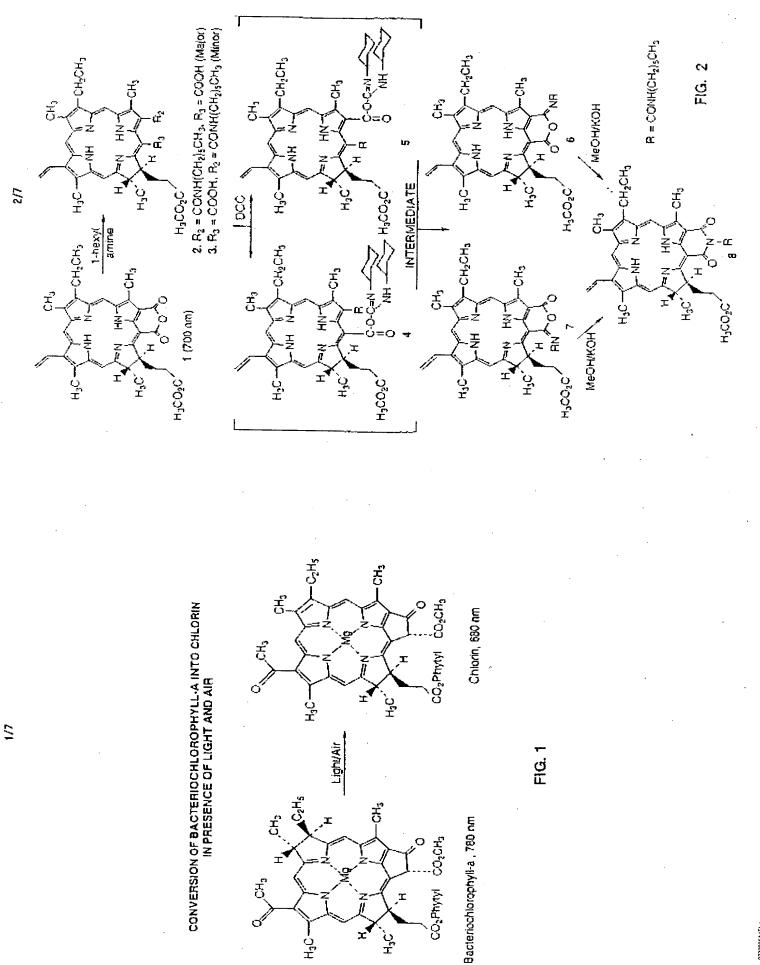
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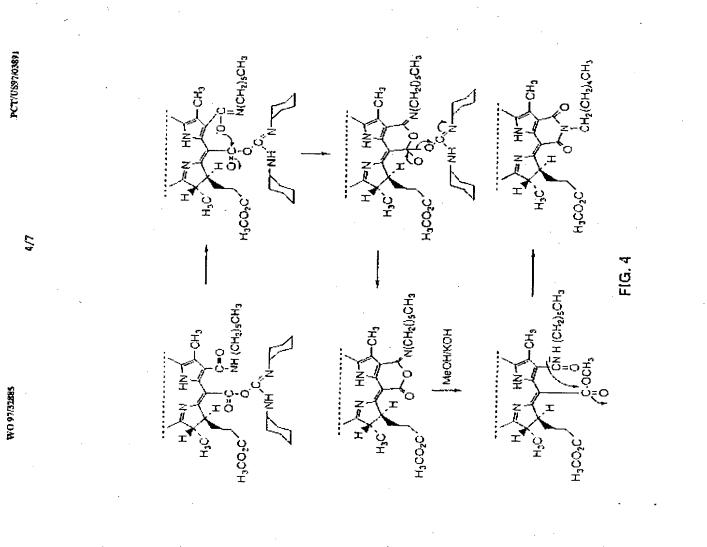
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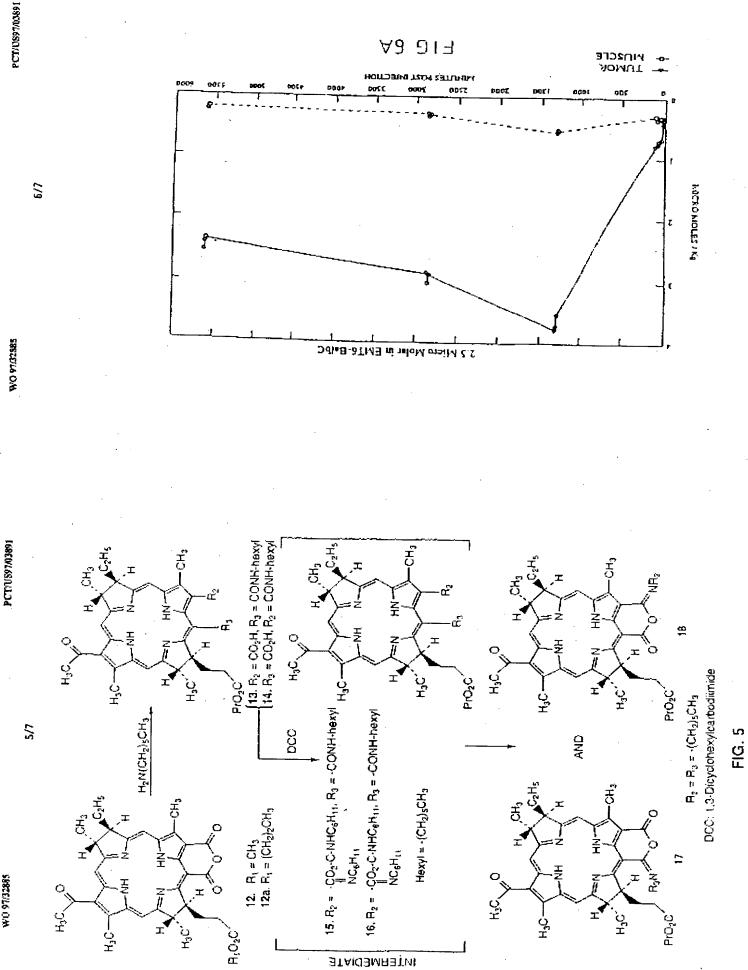
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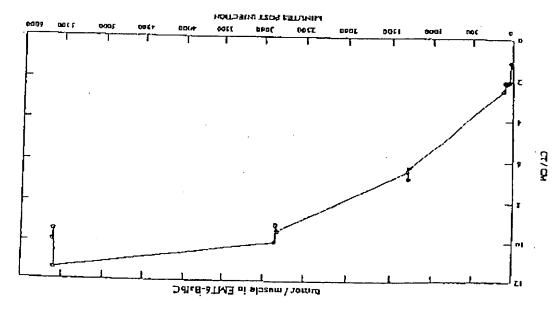
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